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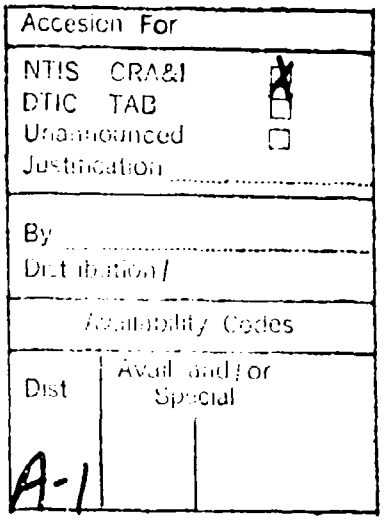

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EXECUTIVE SUMMARY

A. Hazard Assessment

1. Petroleum Smokes

The petroleum smokes, fog oil and diesel fuel, contain highly variable mixes of hydrocarbons which have not been well characterized. The disseminated smokes contain essentially the same class fractions as the bulk materials from which they are generated, although the vapor phases of the smokes are richer in the more volatile components. The smoke particles contain the less volatile components and show a slight increase in the proportion of aromatics to aliphatics than present in the bulk material. This enrichment may be due to oxidation of some of the aliphatic components. The fog oil contains approximately 50 percent by weight of aromatics whereas the diesel fuel has about half this amount. The mass median diameter of the aerosol particles is 1 to 1.5 um, depending on concentration, and are within the deep lung respirable range for humans and laboratory animals.

Continuous skin exposure to petroleum products can produce severe dermal irritation and other dermatoses. The oils and fuels break down the protective defenses of the skin barrier with resultant inflammation, eczemas and infection. Diesel fuel produces more severe dermal toxicity than the lubricating oils which constitute the fog oil smoke. Neither petroleum product elicits skin sensitization responses. The dermal toxicity reactions are not observed when the oils are promptly flushed from skin surfaces with water and proper protective clothing is worn.

Inhalation of petroleum smokes does not produce acute toxicity in animals. Concentrations in the range of 5 to 10 g m^{-3} and 3 to 4 hours exposure produce approximately 50 percent mortality in mice, rats and guinea pigs. For shorter exposure periods, higher concentrations are required to produce mortality. Mortality in acute studies are due to vascular injury, acute inflammatory lesions, epithelial necrosis of the lung, and bronchopneumonia. Surviving animals showed recovery from some acute toxicity responses at 14 days post-exposure. Repeated daily exposures over four to nine week periods at various concentrations, daily exposure durations and frequency of exposures per week demonstrated that the lung was the main target organ for the petroleum smokes' toxicity in rats. Focal accumulations of free pulmonary cells were observed associated with thickening and hypercellularity of alveolar walls, and end expiratory volume and functional residual capacity were increased. These effects persisted for up to two weeks after the end of exposure. The severity of the biologic effects in the diesel fuel exposures were highly correlated with the number of weekly exposures; three days per week exposure produced more severe toxic reactions than one weekly treatment. Six hour daily exposures at concentrations of 1.3 and 2.0 g m^{-3} also produced greater toxic effects than two hour daily exposures at 4.0 or 6.0 g m^{-3}. In the fog oil experiments, concentration was the most important determinant of toxic response (1.5 vs 0.5 g m^{-3}) rather than exposure

duration (1 or 3.5 hours per day) or exposure frequency (2 or 4 days per week).

Longer term exposures of 13 weeks to lower concentrations of the petroleum smokes demonstrated reduced toxicity. Although the frequency of weekly exposures were different for the diesel fuel and fog oil (2 vs 4 weekly exposures), 3.5 to 4 hour daily exposures at 1.5 g m^{-3} for each smoke gave similar results. At the end of the exposure period, histologic sections of lung showed marked hypercellularity in the alveolar region and septa. Lung wet weights were increased indicating an edematous reaction. In diesel fuel exposed rats, all lesions were reversible after an 8 week recovery period. The fog oil treated rats exhibited multifocal granuloma formation 4 weeks after the end of exposure. The development of granulomas after cessation of exposure may be a nonspecific response of the lung to foreign particles when its clearance mechanisms are overwhelmed. At lower concentrations of the petroleum smokes (0.25 and 0.5 g m^{-3}), mild to moderate hypercellularity was observed after the end of exposure but tissue damage was not present.

Epidemiologic studies of non-military workers who are exposed to lubricating oils and oil aerosols have shown increased incidences of skin squamous cell carcinomas but not increased respiratory tumors due to exposure to lubricating oil aerosols. The major health hazard associated with highly refined mineral oils such as fog oils purchased after April 1986 was lipoid pneumonia. Efforts to protect those who may be occupationally exposed an 8-hour time weighted average exposure limit of 5 mg/m^3 (for respirable fraction) is listed in the ACGIH 1992-93 "Threshold Limit Values And Biological Exposure Indices". The evidence for diesel fuel carcinogenicity from laboratory studies is uncertain.

2. HC Smoke

The major component in the HC smoke is zinc chloride ($ZnCl_2$) particles with organochlorine compounds in the gaseous phase comprising approximately 10 percent of the total smoke. Traces of lead and cadmium are also present in the particulate phase. The initial mass median particle diameter is 0.3 um, which increases with concentration and time after detonation.

$ZnCl_2$ is an extremely corrosive material and has induced severe corneal damage in laboratory animals. Severe skin irritation has been produced in rabbits and in humans particularly at sites of recent injury.

There are only a few laboratory studies on the inhalation toxicity of HC smoke. Earlier reports did not specify the concentration and duration of exposures producing mortality. Incidents of accidental human exposure and laboratory studies have shown that lethal concentrations are in the range of 4 to 5 g m^{-3} or greater for single 30 minute exposures. The typical symptoms and clinical signs of exposure to high concentrations are indicative of severe irritation, inflammation, epithelial necrosis and parenchymal infiltration. Manifestations of toxicity can be delayed at lower

concentrations and in some individuals may be slowly resolved. Volunteers exposed to atmospheres containing 120 mg m^{-3} complained of nose, throat and chest irritation, cough and nausea after 2 minutes. After 2 minutes at 80 mg m^{-3}, most subjects had slight nausea, few coughed, and all could smell the smoke.

Although information on pulmonary and skin absorption of zinc in humans remains unavailable, during a previous experiment, 14 pre-terminal patients were given an intravenous infusion of radiolabelled zinc to determine distribution and organ uptake at autopsy. Between 1 and 174 day post exposure, skeletal muscle and the liver showed maxium uptake followed by the spleen, lung, pancreas and prostate. Additional studies in laboratory animals have shown identical patterns of absorption and distribution.

3. Phosphorus Smokes

The phosphorus munitions contain either red or white phosphorus in various matrices: felt, butyl rubber or polymer epoxy binder. The composition of the phosphorus smokes are very similar being composed primarily of polyphosphoric acids with trace levels (less than one percent) of organic compounds. Particle size distributions center around 1.0 um with a narrow range.

Transient eye irritation has been noted in animals exposed for 8 minutes a day. In humans, the primary symptom is upper respiratory irritation. The minimum harassing concentration of phosphorus smokes is about 0.6 to 0.7 g m^{-3} for 2-4 minutes. Respiratory distress, nasal discharge, coughing, and soreness and irritation of the throat were noted. The LC50 in rats for single or five daily one hour exposures ranges from 1.6 to 2.3 g m^{-3}. Since five daily 4 hour exposures to approximately 1.0 mg m^{-3} did not produce deaths, concentration is probably the major determinant of lethality.

Repeated exposures of rats for brief daily periods (15 minutes) to 0.5 or 1.0 g m^{-3} led to the production of laryngitis and tracheitis with some animals at the highest concentration displaying interstitial pneumonia after 6 weeks of exposure. These histopathologic changes were also present after 13 weeks of exposure and were not resolved four weeks after the end of exposures. A concentration of 0.2 g m^{-3} had no effect. Rats given repeated daily exposures for longer periods (2 hours) to concentrations in the range of 0.4 to 1.2 g m^{-3} displayed terminal bronchiolar fibrosis after 4 weeks of exposure. This lesion increased in incidence and severity with increasing concentration and weeks of exposure. The histologic changes did not regress during an eight week recovery period after 13 weeks of exposure. There were indications that pulmonary defense mechanisms are compromised after 13 weeks of exposure. In similar studies, a decline in pulmonary bacterialcidal activity in laboratory rats exposed to the aerosol was displayed.

Mutagenic tests, both in vivo and in vitro, did not provide evidence of a

genotoxic effect for phosphorus smokes.

4. Brass

The disseminated smoke is an irregular flake and contains an alloy of approximately 75 percent copper and 25 percent zinc. The mass median aerodynamic diameter (MMAD) is 2.1 to 2.3 um. This material was not a skin irritant and was only a mild eye irritant after 24 hours. Acute exposures for four hours to concentrations up to 0.2 g m^{-3} produced acute inflammatory responses in the respiratory tract which are reversible. The effects of repeated inhalation exposures are under investigation.

5. Dyes

The dye mixtures in the inventory M18 colored smoke grenades, yellow, green, red and violet, gave positive results in the Ames Salmonella reversion assay. One of the major components in the yellow grenade dye mix, dibenzochrysenedione (Vat Yellow-4), gave positive results in a National Cancer Institute carcinogenic bioassay. A major component of the red grenade and part of the dye mix in the violet grenade, 1-methylaminoanthraquinone undergoes oxidative demethylation during grenade detonation to produce small quantities of 2-aminoanthroquinone which is a suspect animal carcinogen. The major component in the green grenade, 1,4-di-p-toluidino-9,10-anthraquinone (Solvent Green 3) was negative in the Ames bioassay.

The yellow dye now used in the M18 grenade, Solvent Yellow 33, was negative in the standard Ames test strains and an in vivo cytogenetic test but was positive in a mouse lymphoma assay. Repeated inhalation exposure to 234 mg m^{-3} (MMAD: 3-5 um) produced mild toxic effects in rats. The green grenade contains 30 percent of this dye and 70 percent of Solvent Green 3. This mixture gave the same mutagenic results as the Solvent Yellow 33. The green dye is retained in rat lung and elicits a mild inflammatory response.

6. Titanium Dioxide

Generally classified as a "nuisance dust" TiO_2 has been used in several non-military applications to include paints, plastics, and floor coverings. Occupational exposure to TiO_2 has shown no evidence of adverse pulmonary effects. Military application as a training smoke grenade filler is currently under investigation.

7. Graphite

Also classified as a "nuisance dust" graphite is composed predominantly of carbon with a few trace impurities totaling less than 1% by weight. Two synthetic graphite powders are currently in use by the U.S. military (Micro-260 and KS-2).

Exposures, in those occupationally exposed, typically show no adverse effects (during a working lifetime) if exposures are kept below 10 mg m^{-3}. Exposures greater than this may compromise clearing mechanisms resulting in the accumulation of dust-laden macrophages and the proliferation of Type II pneumocytes.

The American Conference of Governmental Industrial Hygienists has issued a notice of intended change to limit graphite exposure (except fibers) to 2 mg m^{-3} of the respirable fraction (ACGIH 1990)

TOXICOLOGY REVIEW

A. Diesel Fuel (DF) Smoke

1. General Information and Properties

a. Bulk material.

Diesel fuel No. 2 (DF-2) is the bulk material that is used in the DF Vehicle Engine Exhaust Smoke System (VEESS). It is a highly variable mixture of hydrocarbons, none of which constitutes greater than two percent of the total mass of the mixture. The performance characteristics of the DF-2 are set forth in Federal Specification VV-F-800C. Detailed analysis[1] of a particular sample of DF-2 used by the USEPA for compliance testing showed the following fractions, obtained by high performance liquid chromatography (HPLC): saturated hydrocarbons, C_9H_{20} to $C_{20}H_{42}$ (70 percent), substituted benzenes (16 percent), 2-ring [illegible] (12 percent), 3-ring aromatics (2 percent), and semi-polar aromatics (0.2 p[illegible]. These proportions may be regarded as typical of DF-2, but the products of differe[illegible]ers and different lots obtained from the same manufacturer may vary considera[illegible]y, even though they meet the specifications.

b. Combustion products.

The DF-2 is not combusted in the process of dissemination as smoke. It is vaporized by introducing it at a rate of about one gallon per minute into the exhaust manifold of an armored vehicle. The temperature of the manifold has been estimated at approximately 600°C, and the exhaust gases contain about 10 percent oxygen, so some oxidation undoubtedly takes place[1] found no significant levels of constituents not found in the unaerosolized DF-2 in the aerosol produced under conditions similar to VEESS production but in a nitrogen atmosphere in which there would not have been any possibility of oxidation. Jenkins and associates were able to obtain some limited samples of VEESS produced at Smoke Week III.[2] The chromatogram of these samples did not show any products in the aerosol that were not present in the unaerosolized DF-2, but there was insufficient sample to perform definitive analyses. In another experiment by Jenkins et al[2], DF-2 smoke was generated in the presence of a 50:50 nitrogen:air atmosphere (approximating the oxygen content of the diesel exhaust atmosphere). There was noted a decrease in the relative proportion of aliphatics in the aerosol, indicating that this fraction was more susceptible to oxidation than were the aromatic fractions. The major change noted in all of the studies was a fractionation of the components on the basis of their relative volatilities, the aerosol particulates being richer in the less volatile components and the vapor phase being richer in the lighter components. This equilibrium is also dependent on ambient temperature and aerosol concentration, with both higher temperatures and dilution favoring transfer of a greater portion of the DF-2 into the vapor phase. The toxicological implications of this fractionation phenomenon are not known. The mass

median diameters of the aerosol particles are in the range of 0.9-1.1 um with a σ_g of 1.4-1.5 um.

2. Pharmacokinetics/Metabolism

There have been no definitive metabolic or pharmacokinetic studies done with DF aerosols. It is assumed[3] that absorption, distribution, and excretion will follow the same general pattern as the constituent hydrocarbons, some of which are metabolized in the liver. Jenkins et al.[4] performed a dosimetry study in which decachlorobiphenyl (DCPB) was used as a tracer for the DF-2 aerosol in single inhalation exposures of 2 and 6 hours duration to aerosol concentrations such that the Ct product was either 8000 or 12000 mg-hr m^{-3}. This was intended only to trace the initial deposition and subsequent removal of DF aerosol from the lung, not as a complete study of the fate of inhaled DF. The fraction of the inhaled particles that was retained in the rats in this study ranged from 4 to 8 percent. The majority of deposition took place in the lungs, but 30 percent of the recovered tracer was found in the digestive tract. Only 1.5 percent of the DCBP was retained in the upper respiratory tract. At each of the Ct levels, animals with longer exposures had proportionately less of the tracer retained in the lung, indicating that changes in uptake and/or clearance and translocation were taking place during the time of exposure (2 to 6 hours).

3. Health Effects

a. Skin and Eye Irritation

Repeated contact with diesel fuel leads to constant skin erythema in sensitive individuals.[3] In a primary dermal irritation test where marketplace diesel fuel was applied for 24 hours, this sample produced severe erythema and edema with blistering and open sores. The sores scabbed over by day 7 with significant healing by day 14. This reaction was considered to fall in the Draize extremely irritating category.[5] This sample of diesel fuel was not an eye irritant and did not elicit skin sensitization in guinea pigs. In a subacute dermal toxicity test, eight rabbits were treated with 4 or 8 mL kg^{-1}. The animals remained bandaged for 24 hours, at which time the patches were removed and a new dose of test material was applied. The dosage regimen was treatment for five consecutive days followed by a two-day rest period and then treatment again for five consecutive days. The diesel fuel produced 0 and 67 percent mortality at 4 and 8 mL kg^{-1}, respectively. Toxic signs at both doses included weight loss with anorexia, depression and signs of severe dermal irritation. Necropsy revealed congested kidneys and livers. Histopathology of the test site revealed acanthosis, acute and chronic inflammation, dermal congestion and edema, dermal necrosis, and parakeratosis. All animals dying on test exhibited signs of chronic dermal irritation, severe anorexia and depression as the test progressed. Dermal irritation with infection, depression and anorexia were the primary causes of death rather than systemic toxicity.

b. Acute Oral Toxicity

The acute oral toxicity rating for diesel fuel to humans is moderately toxic: between 25 and 500 mg.[6]

The LD50 of diesel fuel of unspecified content in Wistar rats has been reported[3] as 16 Ml/kg (range of 6.7-38.4 Ml/kg). A marketplace sample had a LD50 for Sprague-Dawley rats of 9.0 Ml/kg (95% C.I., 5.6-14.5).[5]

c. Acute Inhalation Effects

Toxicity studies conducted to determine the acute inhalation effects of exposure to Grade II (regular) diesel fuel smoke used a smoke generated from the VEESS installed in an M60A1 tank.[7] The smoke was drawn into an exposure chamber to achieve target concentrations and the chamber was then sealed (static airflow conditions). For exposures exceeding 60 minutes, the chamber was refilled with fresh smoke each hour. The concentration level was maintained at 8940 + 3850 mg m^{-3} (total hydrocarbons) and the exposure duration was varied; 15, 60 or 180 minutes. Sprague-Dawley rats and Hartley guinea pigs (10 of each species, 5 per sex) were exposed at each duration. The shortest duration did not produce mortality or morbidity. The 60 minute exposure produced weight loss in both species and 40 percent mortality in guinea pigs. The longest exposure produced 70 and 40 percent mortality in rats and guinea pigs, respectively with significant weight loss. Animals dying during a 14 day post-exposure period showed necrotizing bronchiolitis, pulmonary edema and bronchopneumonia. These lesions were not apparent in 14 day survivors.

In another acute inhalation study, 5 groups of 10 Sprague Dawley rats (5 of each sex) were exposed in a flow-through chamber to a reference grade diesel fuel aerosol produced by a fabricated generator simulating the VEESS smoke-producing conditions.[8] The aerosol concentrations (based on mass of particles) ranged from 2700 to 16000 mg m^{-3} and exposure durations were 2, 4 or 6 hours. The LC50 for a 2 hour exposure was approximately 14000 mg m^{-3}; for a 4 hour exposure between 8000 and 12000 mg m^{-3}; for a 6 hour exposure between 5300 and 8000 mg m^{-3}. Statistical analyses showed that mortality was highly correlated with the Ct product and that a Ct of 8000 mg-hr m^{-3} was the lower confidence limit for the Ct product expected to result in one percent mortality. All deaths occurred within 48 hours post-exposure. Gross observations at necropsy included darkly reddened lungs, clear or slightly blood-tinged fluid in the trachea and occasionally some blood around the external nares. Respiratory frequency was reduced in rats exposed to concentrations of 2000 mg m^{-3} and greater. The calculated RD50 was 375 mg m^{-3}; this number is the concentration of an airborne irritant that produces a 50 percent reduction in breathing frequency.[9]

d. Repeated Inhalation Exposure Effects

An investigation of the toxic effects of repeated inhalation exposures was conducted using the fabricated generator.[10] This study evaluated the relative importance of concentration, daily exposure duration and weekly frequency of exposure on production of adverse biologic effects. Concentration levels were 1300, 2000, 4000 or 6000 mg m^{-3}; durations were 2 or 6 hours; exposure frequencies were one or three times per week for a total of nine exposures. The two Ct products for each exposure frequency were 8000 or 12000 mg-hr m^{-3}. Biologic endpoints were examined two and 14 days after the end of exposure. The main target organ was the lung. The number of lavaged cells was markedly increased shortly after the end of exposure. The majority of cells were neutrophils suggesting an inflammatory response. After two weeks recovery, the number of macrophages remained elevated but the number of other cells returned to control levels. Body weight gain was also reduced in all groups during exposure but this effect was ameliorated after the end of exposure. Biologic effects which persisted up to two weeks after the end of exposure were changes in pulmonary function and lung parenchymal histology. Carbon monoxide diffusing capacity, total lung capacity and vital capacity were decreased; functional residual capacity was increased. In the lung parenchyma, focal accumulations of alveolar macrophages were present in the alveolar walls and alveolar ruptures were present. The severity of these effects was positively correlated with exposure frequency and duration. Rats exposed three times per week, at daily exposures of 6 hours showed the greatest effects.

e. Subchronic Inhalation Exposure Effects

Four groups of 48 rats received two 4-hour exposures/week for 13 weeks to DF concentrations of 0, 250, 750, and 1500 mg m^{-3}.[11] Half of the animals in each group were allowed to recover for 8 weeks, and the other half were killed immediately post-exposure and assayed. There was an immediate loss in weight among all of the exposed animals after the first exposure, and then the growth rate paralleled that of the sham exposed controls during the remaining 12 weeks of exposure. The rats exposed to the lowest concentration, 250 mg m^{-3} rapidly gained weight during the 8-week recovery period. The other exposed groups showed accelerated growth immediately after exposures were terminated, but their growth curves then became parallel to that of the controls, and their final body weights were lower than the controls at the end of the recovery period. The number of lavaged macrophages was slightly elevated immediately post-exposure in all diesel exposed groups, but this effect was not observed at the end of the recovery period. Lung wet weight was increased at the end of exposure to 1500 mg m^{-3} but not after the recovery period. This change suggests a mild edematous response at the highest concentration. There were no significant differences in the pulmonary function tests attributable to exposure to DF-2 at the dosages used in this study, and there was no

significant cumulative toxicity that could be attributed to these exposures to diesel fuel aerosol. Histopathological examination revealed no lesions that could be attributed to inhalation of the diesel fuel, and the incidence and severity of naturally-occurring lesions were not changed by exposure to the DF-2.

f. Reproductive/Teratogenic Effects

There are no data available.

g. Mutagenic Effects

Diesel fuel exhaust particles and nitroaromatic and aliphatic fractions of DP exhaust have been identified as mutagens in various in vitro tests.[12] A sample of diesel fuel containing 23.9 percent aromatics was negative for mutagenic potential in the Salmonella reversion and mouse lymphoma assays, both with and without activation. This sample was positive in a rat bone marrow cytogenetics assay after five intraperitoneal injections of 2.0 or 6.0 Ml/kg, in a dose-related manner.[6]

h. Carcinogenic Effects

Diesel Fuel DF-2 (the same batch of the reference grade fuel as was used in the inhalation toxicity tests[1,8,10,11] gave a significant positive response when tested as a tumor promoter in the SENCAR mouse skin tumorigenesis bioassay system, but gave negative results when tested as a complete carcinogen in the same system.[13] A carcinogenesis study by the National Toxicology Program is in progress.[12] The material under test is marine diesel fuel.

i. Other Observations

Gastritis has been reported in cases of ingestion of DF.[14] There were no reports of effects on humans from wartime usage of DF as an obscurant.[3]

4. Summary

Diesel fuel aerosol has been shown to be an acute respiratory irritant. Dermal application for extended periods produces severe irritation and histopathology. Its toxicity is moderate when taken orally. Repeated biweekly exposures to diesel fuel aerosols for 2-6 hour daily exposures at concentrations of 2000 to 6000 mg m^{-3} produced pulmonary function and histopathologic changes which were not reversible within two weeks after the end of exposure.

There were slight adverse effects-decreased growth rate, a reversible increase in alveolar macrophages, mild edema-from exposure to concentrations up to 1500 mg m^{-3} twice per week for 13 weeks. There were no histopathologic effects attributable to these exposures.

There are low concentrations of known carcinogens such as benzo(a)pyrene, in diesel fuel. A test for complete carcinogenesis in the SENCAR mouse was negative, but DF-2 was shown to be a promoter of skin tumors in mice which had previously been initiated with a carcinogen.

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B. Fog oil

1. General Information and Properties

a. Bulk Material

Smoke Generator Fuel (SGF) No. 2 oil is the bulk material that is used in the M3 and the XM56 smoke generators. It is very similar to S.A.E. 20 motor oil without additives, and some industrially used lubricants and cutting oils. The performance characteristics of SGF-2 are set forth in the Nato Code No. F-62 and the military specification MIL-F-12070C, Admendment 2. An analysis of a particular sample of SGF-2 oil showed the following fractions obtained by high performance liquid chromatography (HPLC): aliphatics (42.5 percent), aromatics (50 percent), esters (4 percent), alcohols (3 percent), and acids (0.5 percent). These proportions are typical of SGF-2, but the products of different refiners and different lots obtained from the same manufacturer may vary considerably, even though they may meet the specifications.[1]

b. Smoke Aerosol Composition

The SGF-2 is not combusted in the process of dissemination as smoke. It is vaporized by introducing it at a rate of about 0.75 gallons per minute into the engine tube of the M3 generator while the pulsejet engine is in operation. The high temperature of the gases (~600°C) vaporizes the fog oil. The vaporized fog oil is forced through three outlet nozzles into the atmosphere. As the oil vapor emerges from the nozzle at high velocity, large volumes of air are sucked into the vapor stream by the rushing vapor. The resulting dilution and cooling produces an enormous number of condensation nuclei. The whole process depends upon a high temperature followed by quick cooling. In addition to the vaporization of fog oil in the XM56 IR Screening Module graphite is introduced as a powder into the atmosphere where it mixes with the oil mist creating the aerosol cloud.[16] The final oil smoke cloud is stable, and the life of the cloud is determined almost solely by meteorological conditions.

In laboratory experiments by Katz et al.[1], chemical analyses of the oil fogs were performed based on liquid and gas chromatography fractionation and gas chromatography and mass spectrometry identification. The gas chromatography traces consistently indicated the presence of very large numbers of unresolved components with a relatively small number of identifiable structures. Oil fogs were separated into class fractions of aliphatics, aromatics, alcohols, acids and esters. The aliphatic and aromatic fractions predominated in the oil fogs, at 95-99% of the total. In general, the aliphatic, aromatic and ester fractions of the oil fogs appear to parallel the parent oil compositions, suggesting only moderate alteration in the fogforming processes, with however, a tendency toward a slightly increased aromatic content in the oil fogs.

The aliphatic fractions contained identifiable straight and branched chain saturated hydrocarbons in the C_{14}-C_{32} range. The aromatic fractions were in a similar molecular weight range but many more species were identified including substituted benzenes, naphthalenes, anthracenes, phenanthrenes, fluorenes, phenalenes, ionols and others. No cyclic structures beyond tetracyclic groups were observed among the identifiable compounds. A considerable number of nitrogen base materials were identified in both the oils and oil fogs, including quinoline, benzoquinoline and indole derivatives. It should be noted that these nitrogen compounds are present at parts per million levels. The several hundred species identified with reasonable assurance were only a small fraction of the total number which were resolved but not identified or which could not be detected against the massive background of unresolved material.

The oil fogs have been examined to determine size and stability. Size determinations indicated a mass median diameter of 1.16 um with a standard deviation of 0.14 um. On the basis of single particle counting, a bimodal distribution was observed with high levels of submicron particles. The aerosols were relatively stable; after aging for one hour, typical systems showed median particle size increases of the order of 12 percent with a decrease in the mass of suspended particles of about 30 percent.[2]

Since there is limited data on SGF No. 2 toxicity, published studies on automotive and industrial lubricating oils, white and medicinal mineral oils were included in this review. It must be emphasized that lubricating oils may contain additives absent from the SGF No. 2 and thus may differ in their toxicity.

2. Pharmacokinetics/Metabolism

Oil aerosol droplets which are inspired are absorbed by the lung and phagocytized by macrophages.[2] Inhaled oil may be expectorated, removed from the lungs by lymphatics, or encysted in granulomas. Large deposits of oil can accumulate in the lungs as an oil pneumonia after inhalation of an oil aerosol. The polycyclic aromatic hydrocarbons (PAH) present in lubricating oils may be metabolized in the liver to epoxides, which are subsequently converted to phenols and trans-hydrodiols; these may be conjugated with glutathione and metabolized to mercapturic acids for urinary excretion.

3. Health Effects

a. Skin and Eye Effects

No appreciable eye, nose, or throat irritation was reported following the exposure of 7 healthy subjects to up to 40 mg m^{-3} of fresh automotive lubricating oil aerosol for a period of 10 minutes.[2] This was the only report of acute human exposure in the literature. Oil folliculitis, acne, eczema, contact sensitivity, as well as

warts, and premalignant skin conditions (pigmentary changes, atrophy, hyperkeratosis, telangiectasia) have been reported due to chronic exposure to lubricating oils. The back of hands, forearms, and groin are often affected. The lubricating oils break down the protective defenses of the skin. The skin barrier varies in thickness, and oils have less difficulty penetrating thin scrotal skin than the thick skin of the palms of the hands. Eczemas, inflammations, and other conditions occur once the barrier is penetrated. Poor occupational and personal hygiene may be the major factors in production of dermatoses.

Monkeys exposed to automotive lubricating oil aerosols, in concentrations of 132 mg m^{-3}, for 30 minutes of every hour of every day for up to 100 days developed oily fur, leading to thinning and bald areas over the abdomen, head, and back. The fur returned to normal after discontinuation of oil exposure.[1] Hyperkeratosis occurred in Holstein-Friesian calves which were administered white lubricating oil to the skin for 8 weeks, as well as on guinea pigs treated every other day for 4 days with mineral oil or lubricating oil. Lubricating oils were acanthogenic in guinea pig skin. Liquid petrolatum increased the cutaneous reactivity of the skin of albino guinea pigs previously sensitized to 2,4-dinitrochlorobenzene. Erythema, scaling and hyperkeratosis, acanthosis, and hypergranulosis were described.

b. Respiratory Effects

There are numerous case reports of humans with pulmonary lesions, such as granulomas and pneumonias, following oral or repeated nasal administration of food or medicinal grade mineral oils.[4,5,6]

Scattered case reports suggest that occupational exposures to mineral oils may also cause pulmonary lesions.[7,8] In brief, short exposures to high concentrations of mineral oils can be tolerated by animals, but repeated exposures may have debilitating effects. For example, Shoshkes found only scattered alveolar macrophages after single 2-hour exposures of mice to high concentrations (4300 mg m^{-3}) of mineral oil mists. Moderately severe foreign body reactions and occasional patches of lipid pneumonia followed 4-week exposures to the same concentrations.

Lushbaugh found that monkeys were particularly susceptible to the effects of lubricating oils. Exposures to SGF-1 oil at concentrations equaling 63 mg m^{-3} caused minimal effects in rabbits, rats and mice after one year but caused pneumonia and pneumonitis in monkeys after as little as 44 days.[9] Selgrade et al.[10] and Grose et al.[17] observed after 13-week exposures to 1500 mg m^{-3} fog oil. Progressive pulmonary lesions were observed after 13-week exposures to 1500 mg m^{-3} fog oil. Although lubricating oils may vary in their relative toxicity,[9] both conventionally-refined oils equivalent to "old" fog oil[11,9,12] and highly refined mineral oils comparable to "new" fog oil[12,13] can cause pneumonia and pneumonitis. It is not known whether SGF-2 will cause similar toxicological effects.

A no-adverse-effect-level (NOAEL) was cited in only one animal study. Wagner showed that 1- to 2-year exposures to 5 mg/m^3 caused only occasional alveolar machrophages in the five species examined.[13] Adverse effects were observed with the lowest doses used in anll other animal studies. To err on the safe side, the human response whould be assumed to resemble that of the most sensitive animal species. Thus, these data indicate that the permissible exposure level must be considerably less than 63 mg m^{-3} (the lowest-adverse-effect-level) to avoid lipoid pneumonia. Based on the NOAEL of 5 mg m^{-3} and the report that humans experience discomfort at mineral oil concentrations greater than 5 mg m^{-3},[14,13] it is recommended that an 8-hour TWA exposure limit of 5 mg m^{-3} (for the respirable fraction) be adopted by the U.S. Army. This is also where fog oil mists become visible.[16] Some flexibility is inherent in this exposure concentration since most smoke blowing exercises are limited to 4-hour periods. Exposures encountered during this time would be normalized to an 8-hour day which would effectively increase allowable exposures to twice the exposre limit. To prevent excessive exposure, excursion levels as defined by the ACGIH[1] must be observed.

Measurements taken by Young et al.[15] during "operate and maintain" exercises at the U.S. Army Chemical School indicate that more than 50 percent of the cadre and students alike receive exposures greater than 5 mg/m^3 when 1 hour exposures are averaged over an 8-hour period. Young pointed out that these exposures can be reduced by altering work habits and conditions (e.g., leaving the immediate vicinity of the smoke generators except when absolutely necessary). He noted that smoke generators used in these individual training exercises were deployed closer together than would be expected in combat and most unit training circumstances and postulated that reducing the number and proximity of generators would substantially reduce exposures. The introduction of such changes may suffice to reduce exposures below the 8-hour TWA exposure level of 5 mg/m^3. However, masking is essential in those instances where work is performed in the immediate vicinity of the smoke generators. Half-face masks are recommended for such occasions.

b. Acute Inhalation Effects

Liquid petrolatum aerosol at a concentration of 4500 mg m^{-3} for 2 hours was lethal to 2/6 albino mice within two days following exposure. Lubricating oil and white mineral oil aerosols, inhaled by six albino mice, in concentrations of 4330–4500 mg m^{-3} for 2 hours, produced the following pulmonary changes: oil retention in terminal bronchioles and alveolar ducts; vigorous and immediate oil phagocytosis which was complete in 48 hours; no inflammatory response; and no deaths. Albino mice exposed to these oil aerosols for about 90 hours developed marked oil retention in all divisions of the respiratory tree. Coalescence of oil into giant droplets was noted, with the majority of the oil being intracellular, and occasional areas of oil pneumonia. Twenty percent of these animals died from acute lipoid pneumonitis.

Albino mice were exposed to 200 mg m^{-3} of lubricating or mineral oil aerosols

for 4 hours. Hyperplasia of the tracheobronchial epithelium was found in mice sacrificed from 18-144 hours following the exposure. Alveolar macrophages increased in number up to 96 hours following exposure, but the response was "very mild". No oil pneumonias were observed. Mice exposed for 7 hours a day for 4 days to the same concentration showed no pulmonary lesions within 96 hours of the last exposure.

Sprague-Dawley rats were exposed for 2, 3.5, or 6 hours to a SGF No. 2 fog oil smoke aerosol ranging from 1460 to 11020 mg m^{-3}. Animals which died or were terminated in a moribund condition showed changes in the lungs indicative of vascular injury. Animals examined after a 14-day recovery period showed minimal or no histopathological changes, indicating reversibility of the vascular lesions. Rats which died, were moribund, or were terminated 14 days post exposure, showed accumulation of histiocytic macrophages in the peribronchial lymph nodes. For the 2 hour exposures, a LC50 was not determined since the highest concentration that could be generated produced less than 50 percent mortality. The results from the 6 hour duration were too variable. The LC50 for the 3.5 hour exposures was 5190 mg m^{-3}.

c. Repeated Inhalation Exposure Effects

Male Wistar rats exposed to mineral oil aerosols, 30,000 mg m^{-3} for 6 hours a day for up to 3 weeks, developed focal oil granulomas characteristic of a foreign body type of response to the oil in the lungs, as well as classic lipoid pneumonia.

Male and female Sprague-Dawley rats were exposed by inhalation to SGF-2 fog oil. The 4-week exposure regimen consisted of two exposure concentrations (500 and 1500 mg m^{-3}); two exposure durations (1.1 and 3.5 hrs per day); two exposure frequencies (2 and 4 days per week). The average MMAD was 1.0 to 1.3 um. Numerous biological parameters were investigated to determine the health effects of exposure to the fog oil. The pulmonary parameters included: pulmonary function, pulmonary edema, lung weights, lung cell differentials, lung histopathology, and alveolar macrophage phagocytic function. Systemic parameters included: xenobiotic metabolism, histopathology, clinical chemistry; hematology; behavioral response, cardiovascular physiology, and immunology.

The most severe toxic effects were observed at the highest concentration. Daily exposure duration and frequency per week had no significant effect on toxicity. A significant increase in lung wet weights (wet/dry weight ratio) and lavage fluid protein suggests edema of the lungs in animals exposed to 1500 mg m^{-3} for 4 weeks. A concentration-related increase in lung dry weight was observed and may be due to hypercellularity, increased neutrophil and macrophage populations. Histopathologic examination revealed a multifocal pneumonitis in rats exposed to the high dose for 4 weeks. Lung volumes, lung compliance, and ventilation distribution were not affected by fog oil exposure; however, end expiratory volume (EEV)

increased. This lung volume change may be due to the oil accumulation in the lung.

Additional toxic effects were a concentration-related decrease in mean RBC corpuscular volume and hemoglobin and an increase in erythrocytes. Zoxazolamine-induced paralysis time was reduced at both concentrations. The concentration-related effect on paralysis time may indicate an induction of the hepatic P-448 system, an effect which is consistent with the PAH content of the fog oil. There was an equivocal effect on enhancement of natural killer cells at the highest concentration.

d. Subchronic Inhalation Exposure Effects

Exposure of white mice to lubricating oil aerosols, 132 mg m^{-3}, 30 minutes per hour, 24 hours a day for 100 consecutive days, resulted in accumulations of oil in alveolar macrophages and tracheobronchial lymph nodes. An average of 1.65 mg of oil accumulated in one mouse lung, representing 0.4% of total wet lung weight. Rhesus monkeys exposed to the lubricating oil aerosol on the same dosage regimen did not show large accumulations of oil in the lung. Microscopic examination of monkey lung showed moderate degrees of subacute and chronic hyperplastic panbronchiolitis, granulomas, and diffuse bronchopneumonia.

In rats exposed to 100 mg m^{-3} of mineral oil aerosols for 6 hours a day, 5 days a week, for up to 26 months, progressive oil macrophage accumulations were noted, along with interstitial pneumonitis. Pneumonitis resulted after exposure of rats to lubricating oils in concentrations up to 60 mg m^{-3}, 5 hours a day for up to 6 months Leukocytic infiltrates, connective tissue proliferation, and peribronchial and perivascular lymphocytic infiltrates were described. Other toxic signs were myocardial degenerative changes, decreased leukocyte phagocytic activity, leukocytosis and lymphocytopenia, higher neuromuscular excitability threshold, increased parasympathetic tone, decreased respiratory rate, slower heart rate, lower voltage of EKG peaks, and decreased arterial blood pressure, increased blood neuraminic acid levels, lowered albumin, increased alpha 1, alpha 2, and beta globulins, and lower serum agglutinin titers. These latter changes were indicative of depressed immune reactivity. Guinea pigs and rats exposed to 10 mg m^{-3} of lubricating oil aerosols for 4 hours a day for 5 months had lowered antibody titers to typhoid and paratyphoid vaccine and reduced phagocytic activity of the blood of nonvaccinated animals to non-virulent Staphylococcus aureus.

In a 13-week study, male Sprague-Dawley rats were exposed to SGF No. 2 smoke aerosol at concentrations of 500 and 1500 mg m^{-3} for 3.5 hrs per day, 4 days per week. Biologic parameters were examined shortly after the end of exposure and at the end of a 30 day recovery period. Immediately following the 13 week exposures, toxic effects similar to those following the 4-week exposure were observed. A significant increase in lung dry weight at both concentrations correlated with an infiltration of alveolar macrophages. An edematous response, decreased body weight, and reduced serum triglycerides were observed only at the highest

concentration. Additional pathological findings included peribronchial lymphoid hyperplasia and multiple pockets of macrophage accumulation in the peribronchial lymph nodes at both concentrations and focal hemorrhage at 1500 mg m^{-3}. Zoxazolamine-induced paralysis time was significantly decreased at both concentrations. In addition, aryl hydrocarbon hydroxylase (AHH) activity was significantly increased at both concentrations. The correlation between these two parameters further substantiates the hypothesis that the fog oil induces the hepatic cytochrome P-448 system.

After a 30-day recovery period, significant increases in lung wet and dry weights were still apparent in the 1500 mg m^{-3} group. Histologic examination of lung tissue from animals exposed to both concentrations showed an accumulation of alveolar macrophages and lymphoid hyperplasia. In addition, several of the animals exposed to 1500 mg m^{-3} exhibited multifocal granulomatous lesions. The development of granulomas after the cessation of exposure suggests a progressive lesion.

e. Reproductive/Teratogenic Effects

There are no data available.

f. Mutagenic Effects

Arene oxides of benzo(a)pyrene, present in lubricating oils, are mutagenic in strains of Salmonella typhimurium and in Chinese hamster V79 cells.[1]

g. Carcinogenic Effects

Squamous cell carcinoma of the hands, face and groin (scrotum, vulva) after chronic oil exposure in industry has an occurrence which parallels the degree of refining an oil underwent prior to use.[1] The polycyclic aromatic hydrocarbons present in all but white oils are probably responsible for their carcinogenicity. In one region of France employing 5,000 workers who are exposed to lubricating oils and oil aerosols, 63% of 133 squamous cell carcinoma cases from 1960-1974 were of the scrotum, 30% of the arms or hands, and the remainder were of the face and neck. The scrotal cancer rate was 25 per 100,000, which was 36 times higher than the scrotal cancer rate of the general unexposed population of that area. Most men affected with scrotal cancer in the general population are over 70 years old, and it is considered a very rare disease. Oil-exposed men in metalworking, cotton and jute industries who develop scrotal cancer are in the 40-50 year age group and have had more than 6 years of oil exposure. Studies in Birmingham, England, showed the larynx to be a site of excessive second primaries in occupationally exposed workers. Second primary tumors of the bronchus in men in Birmingham with scrotal cancer due to industrial oil exposure were excessive for men in some oily jobs but not others, leading to the conclusion that other factors, such as cigarette smoking and the wide

prevalence of chronic bronchitis in England may be involved in this selected group.

Pulmonary tumor deaths due to inhalation of industrial oil mists are no greater than for the unexposed populations. Cancer mortality patterns in over 5,000 white males employed in metalworking for at least one year between 1938 and 1967 did not show any exposure-response relationship or latency effect due to oil mist exposure. In some studies, higher bronchial cancer mortality rates have been reported, but the results are questionable due to comparisons with non-age-matched, unexposed populations. The few reports of bronchogenic carcinoma in oil-exposed workers have failed to consider smoking history and other possible eticlogic agents. Medical records from World War II reportedly revealed no indications that smoke generator unit personnel, or armies living in constant smoke screens for weeks on end, experienced any illness or carcinogenic effects related to exposure to fog oil smoke screens. There was no indication that precautions had been taken to avoid exposure. The documentation of exposure and medical follow-up of exposed personnel are unknown.[2]

The CAF1/JAX mouse had a high spontaneous pulmonary tumor incidence after exposures 5 days a week, 6 hours a day for 16 months to 100 mg m^{-3} of mineral oil showed decreased tumor formation compared to unexposed control mice. Benzo(a)pyrene-containing lubricating oil promoted tumor production by 7,12-dimethylbenz(a)anthracene. In 24 mice given 14 applications of 0.25 mL of lubricating oil over 8.5 weeks, 11 developed malignancies. There were 9 squamous cell carcinomas, 1 basal cell carcinoma, and 1 sarcoma. In 24 animals treated with lubricating oil only, 6 animals developed skin tumors in 84 weeks, of which there were 1 sarcoma, 2 carcinosarcomas, 3 squamous cell carcinomas, and 2 benign squamous papillomas after 12 and 13 months.

4. Summary

Airborne oil droplets are not acutely toxic to humans and laboratory animals. The lethal concentrations for SGF-2 fog oil and automotive lubricating oils are in the 5 to 10 g m^{-3} range. Mineral oil aerosols are even less toxic than lubricating oils. Epidemiologic studies have shown that industrial exposures to lubricating oil mists can produce dermatoses and this observation is corroborated by laboratory animal studies. Repeated inhalation exposures of animals to lubricating oil mists in the range of 60 to 200 mg m^{-3} can produce a number of systemic pathologic changes. The primary toxic response in pulmonary tissue is related to the presence of foreign bodies. There is a recruitment of cells in localized areas leading to granulomatous lesions which are only slowly resolved. Other systemic effects reported in the literature are an oil pneumonitis (pneumonia), hepatic and cardiac lesions and immunologic depression. The latter effects may be due to the presence of additives or other contaminants in automotive lubricating oil. Thirteen week exposures of rats, 4 days per week, to SGF No. 2 fog oil at concentrations of 500 and 1500 mg m^{-3}

elicited concentration-related effects in the respiratory tract which were moderate and, except for the progressive granuloma formation, were reversible. The changes in hepatic microsomal activities may have been due to the PAH content of the fog oil and these changes were reversible. Toxic effects on other organs were not apparent.

Human epidemiological data indicate that chronic exposure of the skin to lubricating oil liquids or aerosols increases the incidence of skin cancer in the exposed population. Epidemiological data does not strongly support a significant increase in respiratory tumors due solely to exposure to lubricating oil aerosols. Personal habits, such as cigarette smoking, and genetic predisposition may play important roles in the increased incidence of respiratory cancer among workers exposed to oil aerosols. Only equivocal evidence for enhanced tumor incidences after exposure to lubricating oils by the dermal and inhalation routes has been obtained from laboratory animal studies.

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C. HC Smoke

1. General Information and Properties

a. Bulk Material

Laboratory investigations were conducted on the composition of the materials in the M5 (30 lb smoke pot).[1] Each smoke pot consisted of a canister containing two layers of chemical mixtures of 46% hexachloroethane (HC), 48% zinc oxide (ZnO), and 6% powdered aluminum (Al).

The inorganic impurities in the reagent mixes were principally cadmium (50 to 1500 ppm by weight) and lead (30 to 90 ppm by weight), with traces of arsenic (a few ppm by weight) and mercury (fractional ppm).

b. Combustion Products

Katz, et al. (1980) conducted a study to characterize the physical and chemical compositions of HC smoke and found it consisted predominately of $ZnCl_2$. Hence, field sampling for HC smoke exposure specifically was designed to collect $ZnCl_2$, and samples were quantitatively analyzed for zinc ion concentration.[20] The evaluation of soldiers' exposure to HC smoke, therefore, is based on the TLV of 1 mg m^{-3} for $ZnCl_2$ recommended by the ACGIH.[22] The potential health hazards from exposure to $ZnCl_2$ in HC smoke was studied.[21] Zinc chloride is hydroscopic and stringent. Persons breathing high concentrations will suffer from pulmonary irritation. Extended exposure can be fatal. Hill et al. (1978) cited cases in which workers' exposure to $ZnCl_2$ in the eyes and nose resulted in burns on the eyes, permanently impaired vision, and permanent loss of sense of smell.

The gaseous products of the smoke generating reaction were collected in a simulated field generation and in a series of scaled-down laboratory experiments. The quantitative data from the field trial were compromised because of a long interval between sample collection and chemical analyses. The qualitative data, however, showed that the same gaseous products were formed in both the field test and the laboratory studies.

Gaseous components formed in both laboratory and field reactions were:

	Mass %[1]	Calculated ppm[2]	
	(Laboratory)	(Laboratory)	(Field)
carbon monoxide (CO)	0.6-2.3	9.5	0.1
hydrogen chloride (HCl)	0.02-2.1	9	-
carbonyl chloride ($COCl_2$)	0.10-1.0	1.5	5.5
carbon tetrachloride (C_2Cl_4)	0.30-5.0	7.5	11.5
ethylene tetrachloride (C_2Cl_4)	3.00-17.0	29.5	29.5
exachloroethane (C_2Cl_4)	0.30-5.0	5	24.5
hexachlorobenzene (C_6Cl_6)	0.4-0.9	2	8
chlorine (Cl_2)	Not determined	3.5	-

[1]The smoke pots used in the laboratory studies functioned at about 70 percent efficiency. Mass percent is based on the initial weight of the reaction mixture.

[2]Based on an estimated aerosol concentration of 500 mg m^{-3}.

The aerosol particles were predominantly $ZnCl_2$(410 ppm) with 1 to 2 percent of aluminum chloride and traces of lead (0.25 ppm), arsenic (0.1 ppb), magnesium (0.25 ppb) and cadmium (0.25 ppm). The initial aerosol median particle diameter measured 0.3 um. At a concentration of 1 to 2 x 10^8 particles cm^{-3}, the aerosol is stable for 20 to 30 minutes. Aerosol growth accelerates with increasing concentration.

$$2Al + C_2Cl_6 + 3ZnO \rightarrow 3ZnCl_2 + Al_2O_3 + 2C + Heat$$

(Katz et al. [1980] defined the ideal HC mixture chemical reaction in the above stoichiometric equation)

Zinc chloride ($ZnCl_2$) leaves the reaction zone as a hot vapor in the burning HC smoke pot, and on cooling below the condensation point, forms the desired aerosol. Hence, $ZnCl_2$ is the target substance to sample in HC smoke. Zinc chloride sampling entailed the use of mixed cellulose ester filters connected to a DHFS-113A high flow pump calibrated at 3.5 Lpm, as described above. The sampling train was loaded onto the LCE for personnel sampling.[16]

In another study, the main products from an initial mixture of 44 percent HC, 32-47 percent ZnO, 2 percent potassium nitrate and 7-22 percent calcium silicide were determined to include $ZnCl_2$, carbon, CO and silicon with traces of phosgene.[2,5] For this mixture, 100 g of mixture released a little over 40 g of $ZnCl_2$.

2. Pharmacokinetics

Radiolabelled zinc as the chloride has been administered by intravenous and oral routes.[3] Information on pulmonary and skin absorption of zinc in humans is not available. In one experiment, 14 pre-terminal patients having various malignancies were given an intravenous infusion. The distribution of radiozinc was determined at autopsy which occurred between 1 and 174 days after the infusion. The skeletal muscle and liver showed maximum uptake followed by spleen, lung, pancreas and prostate. The biological half-life of zinc in human liver was about 75 days. A more rapid turnover of the administered dose occurred in the pancreas and spleen. In another study, the liver and kidney had the greatest amount of radiozinc after intravenous administration. Absorption studies in humans after oral administration have shown that radiozinc entered the blood stream rapidly from the gastrointestinal tract and reached a peak in plasma by the fourth hour after ingestion of the dose. The average biologic half-life of orally administered zinc was 154 days.

Following intravenous and oral administration, the main pathway of excretion in humans was via the intestine. Urinary excretion is low and ranged from 0.7 to 2.1 percent during the 15 days after treatment.

Studies in laboratory animals have shown the same pattern of absorption, distribution, retention and excretion. Zinc chloride is poorly absorbed through the skin as shown by percutaneous application studies of radiozinc to guinea pigs; less than one percent was absorbed in 5 hours.

The exposure route for the gaseous components would be primarily by inhalation. Dermal absorption does not appear to be a significant component in total systemic uptake for most of the organochlorine compounds except CCl_4.

3. Health Effects

a. Skin and Eye Irritation

Percutaneous application of zinc chloride on the shaved skin of guinea pigs caused reduced weight gain but had no other toxic effects. Eye application of a concentrated (50%) zinc chloride solution in the eyes of albino rabbits induced severe corneal damage. Corneal opacification became evident four days to two weeks after treatment and the cornea also became ulcerated and perforated. For humans, the development of skin lesions and burns on the hands and fingers of men who handled $ZnCl_2$-treated railway ties has been reported. These lesions generally developed at

the site of a recent injury such as an abrasion, burn, chaffing, or splinter. The severity of the zinc chloride lesions was dependent upon the length of exposure and the size of the antecedent injury.

A simulated environmental deposition HC smoke mixture (500 mg) was applied for 24 hours to abraded and unabraded skin of albino rabbits.

Component	Percent	mg
HCl	1.83	9.2
C_2Cl_4	14.41	72.1
CCl_4	5.52	27.6
C_2Cl_6	4.48	22.4
C_6Cl_6	1.63	8.2
ZnCl	1.07	305.4
Al_2O_3	10.92	54.6
$PbCl_2$	0.45	0.09
$CdCl_2$	0.26	0.05
$AsCl_3$	0.001	0.005

Moderate to severe erythema and edema were visible on both abraded and unabraded skin. By 72 hours, all test sites appeared corroded with the sites black and hardened. This mixture was considered to be a severe skin irritant to rabbits.

C_2Cl_6 produced reversible eye irritation and mild skin irritation in rabbits. CCl_4 produced mild skin irritation at 4 mg and mild eye irritation at 2.2 mg when applied for 30 seconds to rabbits. Application of 500 mg of CCl4 for 24 hours to rabbit eyes produced a severe reaction. C2Cl4 application of 810 mg to rabbit skin for 24 hours produced severe irritation. Application of 162 mg to the rabbit eye produced a mild reaction. C_2Cl_5 produced reversible eye irritation and mild skin irritation.[4]

b. Acute Oral Toxicity

The average oral lethal dose of $ZnCl_2$ for rats was 750 mg kg^{-1}. Necropsies revealed perforation of the stomach and penetration into the liver tissue and pyloric stenosis. In those animals dying early, mucosal damage was less evident but tremor, atopia, dyspnea and a drop in body temperatures were observed. The average oral lethal dose to rabbits was 1000 mg kg^{-1}. Necropsy revealed symptoms similar to those previously described for the rats.

Accidental ingestion of $ZnCl_2$ by humans has been reported. Severe cases of food poisoning have developed in persons who had eaten cooked apples prepared and served in a galvanized iron kettle. Chemical analyses revealed 83 mg of zinc per 100 g of apples. The characteristic symptoms of intoxication include salivation; edema

of the glottis, difficulty swallowing; massive swelling of the lips; pain in the mouth, throat and epigastrium; recurrent violent vomiting; severe abdominal pain; and bloody diarrhea. A rapid but weak pulse, a drop in blood pressure, and cold, clammy skin are usually evident. Muscular weakness or spasms, aphonia, and sensory disturbances may occur in some cases.

c. Acute Inhalation Effects

Acute inhalation exposures to $ZnCl_2$ smoke caused pulmonary irritation in mice and dogs. The dose of $ZnCl_2$ smoke required to kill 50 percent of exposed mice (LCt50) was estimated to be 11,800 mg-min m^{-3}. At 2000 mg-min m^{-3}, macroscopic or histologic lung damage was no longer evident. Five dogs weighing 6-13 kg were exposed to high concentrations for an average of 20 minutes. Hemoconcentration developed after the radiologic appearance of pulmonary edema and returned to normal within a few days. Lung volume increased following exposure but returned to normal as the edema subsided.

Rats and rabbits were exposed to two pyrotechnic mixtures used to generate $ZnCl_2$ smoke in the United Kingdom.[5]

	Percent by Weight			
	HC	ZnO	KNO_3	Other
Composition I	44	32-47	2	Calcium silicide 7-22
Composition II	45	35-50		Silumin 5-20

The animals of each species were randomly allocated to 2 test groups, each numbering 10 animals and a control group of 5. The test groups were exposed to one of the compositions for 30 minutes in a 10 m^3 chamber by igniting the pyrotechnic mixture. The mass of solid material collected through filter papers was 2.75 g m^{-3} (Zn: 0.60 g m^{-3}) for Composition I and 4.00 g m^{-3} (Zn: 0.81 g m^{-3}) for Composition II.

For Composition I, 7 of 10 rabbits died between the end of exposure and 24 hours later; 5 of 10 rats died between 6 and 24 hours post-exposure. Exposure to Composition II produced mortality in all rabbits between 6 hours and one week post-exposure; 5 of 10 rats died between the end of exposure and 24 hours later. Histopathologic changes in animals exposed to the two compositions were qualitatively similar. These changes included inflammation and necrosis of the larynx and trachea; alveolitis; petechial hemorrhages in the lung and edema. Animals surviving 14 days after the end of exposure showed mild to moderate laryngeal and tracheal inflammation and macrophage and lymphocyte aggregations in the alveoli.

There have been no studies that measured soldier exposure to fog oil or HC smoke. Studies have been performed to evaluate the adequacy of experiments to establish a relationship between obscuration and concentration in order to estimate dosage (Policastro and Dunn, 1985)[18] Additional studies have been performed to validate mathematical dispersion models through measurements of total particulate concentration and particle-size distribution (Liljegren et al., 1986b).[19] While these studies will be useful for predicting the probability of exposure at a given distance from the smoke source, they do not have the ability to correlate soldier work patterns (principally affecting the magnitude and the duration of exposure) with smoke plume fluctuations.[16]

The use of military smoke and obscurants in simulated combat training provides realism and allows the soldiers to practice, respond, and operate in an obscured environmnet. A research effort to evaluate the magnitude and extent of soldiers' exposure to smoke during a Military Operations On Urban Terrain (MOUT) training exercise was conducted to identify conditions under which exposures would occur and to derive appropriate recommendations that would prevent or minimize personnel exposure to hexachloroethane smoke. Results indicated that trainees and instructors were exposed to zinc chloride in concentrations ranging from 0.02 to 0.98 milligrams per cubic meter during the 225 minutes of the exercise. Average exposure level was 0.26 milligram per cubic meter with a standard deviation of 0.26.[17]

Most of the reported accidental exposures of humans to $ZnCl_2$ smoke have not specified concentration and/or duration of exposure. Deaths are usually delayed (6 to 18 days); recovery from symptoms occurs one to 13 5 months after the accidents. Inhalation of the smoke is most dangerous in doors or in enclosed spaces with inadequate ventilation. Minor symptoms lasting a few hours have been reported after exposure in the open. Volunteers exposed to atmospheres containing 120 mg m^{-3} of $ZnCl_2$ smoke complained of nose, throat and chest irritation, cough and nausea after 2 minutes. After 2 minutes at 80 mg m^{-3}, most had slight nausea, a few coughed, and all could smell the smoke.

In one case of accidental exposure, air samples were analyzed at the scene of exposure. Three airmen in their late teens developed chemical pneumonitis after exposure to 4075 mg m^{-3} $ZnCl_2$ smoke produced by a grenade. $AlCl_3$ (334 mg m^{-3}), Al_2O_3 (134 mg m^{-3}) and ZnO (108 mg m^{-3}) were also found by analysis. The concentration of the total particulate matter was 800 mg m^{-3} and the average particle size was 0.1 um (range 0.01-25 um). In all three patients, the initial symptoms included nausea, paroxysmal cough, dyspnea and tightness of the chest. Later symptoms, including fever, tachypnea, and cyanosis developed. Coughing became less frequent and nonproductive, but x-rays revealed marked parenchymal infiltration. Vital lung capacity was diminished, but returned to normal. All three patients recovered within one month.

Another recent case of human exposure did not report concentrations but is of interest because of the medical follow-up after exposure.[6] In a 1979 airport disaster drill, exposure to $ZnCl_2$ aerosol occurred due to detonation of a smoke bomb containing HC, ZnO and calcium silicide. Participants experienced upper respiratory tract symptoms which corresponded in frequency of occurrence and intensity with proximity to the site and duration of exposure. The onset of cough and hoarseness or sore throat was characteristic of all early-reported symptoms with the greatest reported prevalence at the time of exposure and a progressive decline thereafter in number of subjects. The late-onset symptoms, nausea, fatigue, or headache, lasted at least 6 hours and occurred from 2 to 20 hours after smoke exposure. In a few exposed individuals, some symptoms such as sore throat or hoarseness, persisted for 5 to 11 days. None of the exposed subjects showed evidence of a pneumonitis from their exposure.[6]

The one-hour LC50s for HCL are 1108 (mouse) and 3124 (rat) ppm. The 30 minute LCLos are 4416 (rabbit and guinea pig) and 1300 (human) ppm. Exposure of men at 50-100 ppm vapor for one hour was barely tolerable, 35 ppm caused irritation of the throat on short exposure and concentrations above 5 ppm are immediately irritating when inhaled. The LC50 for CCL_4 is 9526 ppm (mouse, 8 hr). The LCLos are 1000 (human), 4000 (rat, 4 hr), 14620 (dog, 8 hr), 38,110 (cat, 2 hr), and 20,000 (guinea, 2 hr) ppm. The LCLos for C_2Cl_4 are 4000 ppm (rat, 4 hr) and 3382 ppm (mouse, 2 hr). The LCLos for man are 96 ppm (7 hr), 280 ppm (2 hr) and 600 ppm (10 min). Humans exposed to C_2Cl_4 longer than 1.5 min at 2000 ppm would become unconscious while exposure at 500 ppm for 50 min causes increased salivation, metallic taste, eye irritation, and tightness of the frontal sinus. Exposure for 2 hours at 216 ppm causes light-headedness, burning in the eyes, congestion of the frontal sinus, thickness of the tongue and difficulty in motor coordination. Slight eye irritation can occur after 4 hours exposure at 100 ppm. The LC50 for $COCl_2$ in humans is 400 ppm (2 min). The LCLos in other species are 50 (rat, 30 min), 8 (guinea pig, 20 min), and 80 (dog, 30 min) ppm.

d. Repeated Inhalation Exposure Effects

There are no reports on the effects of repeated exposure to $ZnCl_2$ or HC smoke aerosol.

Repeated exposure of animals to HCl at a concentration of about 34 ppm caused no immediate effects and no morphologic changes attributable to exposure.[7]

Exposure of guinea pigs, rats, dogs and monkeys to 10 ppm CCl_4 for several weeks or months produced detectable accumulation of fat in the liver. This effect was observed in guinea pigs exposed at 5 ppm but not in other animals. There were no effects observed at 1 ppm. Workers exposed to 33 to 124 ppm became fatigued within 2 hours of starting work. Workers exposed to 45-97 ppm reported headache and giddiness. Symptoms of liver dysfunction also occurred. Volunteers exposed to 10-11 ppm for 180 min did not show effects on liver function. Chronic exposure levels which have been associated with reports of illness have been in the range of 5-115 ppm. The lowest average exposure which produced symptoms of liver dysfunction was 20 ppm.[7]

Rats were exposed to C2Cl4 for 8 hr/day, 5 days/week for periods up to 7 months to 70, 230, and 470 ppm. All animals survived with their growth comparable to controls. At 230 and 470 ppm changes were seen in the liver and kidney. On a dosage regimen of 7 hr/day and 5 days/week, rats survived a concentration of 1600 ppm but drowsiness and depression were observed in the first week with enlarged livers and kidneys apparent after four weeks. At 400 ppm, 130 seven-hour exposures over 6 months caused no effects in rats, rabbits or monkeys, while guinea pigs had increased liver and kidney weights and slight fatty degeneration. Fatty degeneration was also observed in the livers of mice exposed 4 hr/day for 1, 2, 4, or 8 weeks to 200 ppm. In humans, prolonged exposure to 200 ppm results in early signs of CNS depression; there is no response to repeated 7 hr/day exposures to 100 ppm.[7]

Rats exposed to 5900 ppm C_2Cl_6 for 8 hours showed severe toxic signs and death. No toxic effects were observed when the dose was reduced to 260 ppm. Dogs developed tremors, ataxia, hypersalivation, severe head bobbing and facial muscular fasciculations when exposed to 260 ppm, 6 hr daily, 5 days/week for six weeks. No effects on body weights, gross necropsy, and organ weight changes were found in rats, quail, pigs and dogs exposed to 15 ppm. Exposures to 48 ppm produced minimal toxic effects.[7]

Exposure of goats, cats, rabbits, guinea pigs, rats and mice to 0.2 ppm of $COCl_2$ 5 hours per day for 5 consecutive days caused evidence of pulmonary edema in 41 percent of the animals and extensive lung lesions were present in 4 percent of the animals. Exposure of these species to 1 ppm in the safe exposure regimen depressed ciliary function and caused serious lung lesions. Guinea pigs treated with low doses of 2.5 ppm for 10 minutes a day for 7 days became relatively resistant to toxic levels of 35 ppm. Repeated exposure of cats to 1.5-3.5 or 5-6 ppm for 10 minutes every day caused no greater damage after 40 days than after 2 days. The

effects of chronic low doses of $COCl_2$ upon the lungs of animals and man in situations where there are no acute symptoms are not known. The National Academy of Science lists a 90 day atmospheric limit for use in submarines of 0.05 ppm.[7]

e. Reproductive/Teratogenic Effects

Ingestion of dietary $ZnCl_2$ (diet contained 2.5 to 5 g kg^{-1}) had no toxic effect on the reproduction and offspring of rats.

Pregnant rats exposed to 300 and 1000 ppm CCl_4 showed significant weight loss and liver damage. Fetuses showed a significant increase in microscopic skeletal abnormalities and decreased weight and end length.

Pregnant rats and mice were exposed to 300 ppm C_2Cl_4 on days 6-15 of gestation without teratologic effect on the offspring although fetotoxicity was evident. No reproductive effects were seen in the offspring of pregnant rats exposed to 100 or 900 ppm.

C_2Cl_6 at dosages toxic to dams, 260 ppm, resulted in only a slight slowing of fetal development.

Specific fetal abnormalities have been noted after oral doses of 40 to 6450 mg/kg C_6Cl_6 to rats. The toxic oral dose for pregnant mice was 1000 mg kg^{-1}.

f. Mutagenic Effects

The mutagenicity of $ZnCl_2$ has been evaluated in two in vitro assays. The infidelity of DNA synthesis was determined in a reaction mixture containing a DNA polymerase, a template-primer of restricted base composition and complementary and noncomplementary deoxynucleoside triphosphates each labeled with different radioactive isotopes. From the ratio of radioactive substrates incorporated, the error frequency was calculated. $ZnCl_2$ had no effect on error frequency, whereas eight metal compounds shown to be mutagens or carcinogens enhanced the infidelity of DNA synthesis (Ref. 8). In another test, 38 metal salts were examined for their capacity to enhance transformation of Syrian hamster embryo cells by a simian adenovirus, SV7. With $ZnCl_2$ and $ZnSO_4$, enhancement was observed in only 3 of 6 and 3 of 7 trials. The authors stated that the results were inconclusive.[9]

The mutagenic effects of HCl were examined in an microsuspension assay utilizing Escherichia coli indicator strains for the detection of chemically-induced preferential kill of repair-deficient strains. Although HCl produced a marked growth reduction of WP2 uvrA, this effect was considered due to toxicity and not to DNA-damaging effect since the remaining WP2 deficient strains which also carry the uvrA mutation gave no indication of preferential kill.[10]

g. Carcinogenic Effects

There are a few studies which purport to demonstrate the carcinogenic effect of Zn. The method of administration (intratesticular or subcutaneous) may have influenced the production of sarcomas by a physical irritant effect. Reviews of this data and other negative studies have lead to the conclusion that there is no adequate evidence that Zn salts are carcinogens.[11]

Several cases of liver cancer in humans after exposure to CCl_4 have been reported. In all of the human cases, the patient had been acutely overexposed to CCl_4. Life time feeding studies in rodents have demonstrated significant excesses in tumor incidence in treated animals.

In an NCI bioassay, gavage of C_2Cl_4 produced hepatocellular carcinomas in mice but not in rats. The NTP is conducting a carcinogenesis bioassay with this compound.[12]

A NCI bioassay showed that B6C3FI mice developed hepatocellular tumors after C_2Cl_6 treatment but no effects were observed in Osborne-Mendel rats.[13] A NTP carcinogenesis bioassay is in progress. NIOSH recommends that C_2Cl_6 be treated as a carcinogen.[14]

An IARC review of data on C_6Cl_6 concluded that this compound was a suspected human carcinogen and a positive animal carcinogen.[15]

4. Summary

Accidental exposures of humans to HC smoke have conclusively demonstrated the marked toxicity of the combustion products aerosol. Most investigators have attributed this toxicity to the major reaction product, $ZnCl_2$. This chloride is known to be very corrosive and the symptoms demonstrated by subjects suggest that the upper respiratory tract is most affected by exposure. Deposition of the aerosol in the upper respiratory tract may be a function of its hydroscopic nature and aerosol particles may rapidly increase in size in the humid pulmonary tract. However, respiratory tract symptoms have also been observed (chemical pneumonitis), particularly at estimated "high" concentrations. The lower limit of detection of HC smoke by humans is approximately 40 mg m^{-3}; a metallic taste has been described. At double this concentration (80 mg m^{-3}) toxic signs are evident. Lethal concentrations are estimated

to be in the g m^{-3} range; duration of exposure less than 30 minutes. Recent animal studies have shown 50 to 100 percent mortality at 3 to 4 g m-3 for 30 minutes. The characteristic features of HC smoke toxicity are late onset of some symptoms and the slow recovery. There is a paucity of information regarding the health hazards of repeated exposure to concentrations of HC smoke which do not produce morbidity.

Although the total amount of the organochlorine compounds represents only 10 percent of the HC smoke, a number of these compounds are suspect or positive animal and human carcinogens. There is no information on the potential carcinogen risk when humans are exposed to mixtures of these chemicals along with $ZnCl_2$. Some investigators have proposed that toxic damage to a tissue or organ may enhance the oncogenic potential of other materials which, by themselves, would not induce neoplasia. It is possible that the corrosive action of $ZnCl_2$ could predispose parts of the respiratory tract to the carcinogenic action of the organochlorine compounds. It is also possible that there may be an inhibitory effect of the total organochlorine mixture as has been demonstrated for some petroleum fractions. As stated above, there is no data base upon which to evaluate these hypotheses. Additionally, no animal laboratory studies have been performed on the HC combustion product mix.

There have been no studies which measure soldier exposure to HC smoke. Efforts have been made to establish a correlation between percent obscuration or cloud density and most probable dose concentration. However, effects on soldiers' performance, at specific distances from the smoke source, are unpredictable.

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D. Phosphorus Smokes

1. General Information and Properties

a. Bulk Material

(1) White Phosphorus in Felt (WP/Felt)

WP/Felt Smoke material is incorporated into the M825 155 mm artillery projectile. WP/Felt consists of 80% white phosphorus impregnated in a wool felt matrix material.[1] The characteristics of the white phosphorus is specified in Military Specification: MIL-C-215B dated 21 August 1969.

(2) Red Phosphorus Butyl Rubber (RP/BR)

RP/BR smoke material is incorporated into the L8A1 smoke grenade used on armored vehicles for self-protection. There are currently 226,000 L8A1 grenades in the inventory.[2] This munition, which ignites while airborne, is designed to obscure vehicles from which grenade clusters are launched. The obscurant portion of the L8A1 grenade consists of 360 grams of a 95 to 5 mixture of red phosphorus and butyl rubber formulated in granules.[3,4] These grenades were originally obtained by the U.S. Army from Great Britain and were then produced at Pine Bluff Arsenal, Arkansas (PBA) until 1985, after which time their production was discontinued. In 1987, production began on the L8A3 grenade, a modification of the L8A1 which employs silica instead of talc as the granule coating material, to reduce phosphorus clumping thereby improve its burning qualities.[5] During grenade production, red phosphorus is plasticized with butyl rubber in an organic solvent and extruded. The resulting granules are dried, pressed into pellets, and then inserted into the rubber sleeve of the L8A3 grenades per month could be produced at PBA.[6] The RP/BR material is prepared by mixing 95 percent oiled red phosphorus with 5 percent butyl rubber in the presence of methylene chloride. The material is extruded into pellets and the methylene chloride is removed by low temperature drying. The RP/BR mixture is softened with hexane vapors (7-8 percent) to facilitate extrusion in the laboratory smoke generator.[1] The characteristics of the oiled red phosphorus is specified in Military Specification: G-92-50 DOD-P-51463(DA) dated 12 February 1980. Sources of RP are becoming more scarce with the last North American manufacturer shut down in 1993. The only available source at this time is The People's Republic Of China.

(3) Red Phosphorus and Nitrate ($RPNO_3$)

Red phosphorus is also the major smoke-generating ingredient in the SM819 mortar round. In this munition, it is compounded with sodium nitrate and an epoxy binder in an approximate ratio of 80:14:6 parts by weight, respectively.[7] Fill for the mortar rounds are prepared by blending powdered red phosphorus, sodium nitrate,

and epoxy binder with appropriate curing agents and solvents, granulating the resulting slurry, and pressing it into wedges for loading. During loading, mortar rounds receive 28 wedges weighing approximately 43 grams each. The munition is designed so that following its detonation, ignited wedges will disperse within the air and on the ground.[7] Consideration is also currently being given to the design and production of a third red phosphorus munition, the XM803 howitzer round, for even larger scale smoke generation.[8]

b. Combustion Products

The combustion products of the WP/Felt, RP/BR and $RPNO_3$ are all very similar in composition. They are composed primarily of ortho-, pyro-, tri-, tetra-, and polyphosphates, with organic compounds and inorganic gases only at trace levels.[1,9,10] The species of phosphoric acids and their relative proportions are similar among the three phosphorus smokes. The organic compounds levels in the three smokes are: 30 ug organic carbon per gram phosphoric acid in RP/BR; 140 ug in RP/N03; 400 ug in WP/Felt. Aerosol particles sizes were within the respirable range, 0.4 to 1.0 um, depending on generation conditions and aerosol age.

2. Pharmacokinetics/Metabolism

There is no data available for the phosphorus smokes.

3. Health Effects

a. Skin and Eye Irritation

Transient ocular irritation has been reported in Sprague Dawley and Fischer 344 rats exposed to RP/BR smoke aerosol concentrations of approximately 21 mg m^{-3} and 165 mg m^{-3} for 8 minutes/day, 5 days/week for 12 weeks. The ocular irritation lasted for approximately one week during the exposure and then subsided as the exposure continued to completion for 12 weeks.[11]

In an additional study outlining the consequences of increased environmental burdens of acid and phosphate deposition, as well as, toxicological effects little if any significant toxicity appears to be associated with elemental red phosphorus unless it is contaminated with the white allotropic form.[12] When applied under patches to the intact or abraded skin of rabbits at doses of 0.5 g per site, no dermal irritation was noted following 24 hours of exposure to red phosphorus. Similarly, dermal application of the element to guinea pigs resulted in no skin irritation or sensitization responses, and interdermal injection resulted in only slight irritation with no skin sensitization. Doses of 100 mg did not result in rabbit eye irritation. Even at oral doses of 10,000 mg/kg, lethality in Fischer 344 male and female rats was less than 20% of the experimental subjects after 14 days, and the oral LD50 was reported as being greater than 10 g kg^{-1}.[13]

b. Acute Inhalation Effects

(1) WP/Felt

A concentration of 1000 mg m^{-3} of white phosphorus smoke is intolerable to human subjects. The minimum harassing concentration of white phosphorus smoke is about 700 mg m^{-3}. Respiratory distress, nasal discharge, coughing, and soreness and irritation of the throat were noted in subjects exposed to about 600 mg m^{-3} for 2-4 minutes. Irritation of nose and throat and coughing were observed in men exposed to levels up to 500 mg m^{-3} for 16 minutes. The symptoms disappeared in all the cases three days post-exposure.[14]

When mice were exposed to white phosphorus smoke at a concentration or 110-900 mg m^{-3} for one hour, there were no deaths during the exposure, but about 20 percent of the animals died 24 hours to 10 days later. At concentrations of 1230 mg m^{-3} five out of 20 animals died during a one-hour exposure, while at 1690 mg m^{-3}, fourteen out of 20 animals died during the exposure. Death in all cases was due to respiratory failure. Other effects observed were hemorrhagic lungs and occasional cloudy swelling of heart, liver and kidney cells.[14]

Exposure of groups of ten rats to 4530 mg m^{-3} for one hour produced one death during exposure. One death occurred 24-48 hours after exposure to 1350 mg m^{-3}. At concentrations of 4460-4810 mg m^{-3}, all the rats died 1-10 days post-exposure. Necropsy findings included pulmonary congestion, edema, occasional atelectasis and cloudy swelling of hepatic and renal cells.[14]

White phosphorus smoke at levels ranging from 540-4810 mg m^{-3} for one hour did not cause any deaths in goats up to 10 days post-exposure. At concentrations ranging from 5230-7310 mg m^{-3} about 3-6 out of 10 goats died 5-10 days post-exposure. There were some deaths in the animals 24 hours postexposure when the concentration was increased to 7750-11470 mg m^{-3} for a one hour exposure. Necropsy findings were pulmonary edema, atelectasis and pneumonia, cloudy cell swelling, and congestion of the liver and kidneys.[15]

Sprague Dawley rats were exposed for 60-90 minutes at concentrations of 505-2018 mg m^{-3}. Hartley guinea pigs were exposed for 5-60 minutes to concentrations of 88-801 mg m^{-3}. The animals showed severe respiratory distress prior to death. The calculated LC50 for the rats was 1569 mg m^{-3} (one hour) and 532 mg m^{-3} (10 minutes) for the guinea pigs.[16]

(2) RP/BR

Sprague-Dawley rats were exposed to RP/BR aerosols for 1 hour daily on 5 consecutive days at concentrations of 1560, 1990, 2490 and 3050 mg m^{-3}.[17] Mortality ranged from 5 to 90 percent with decreased survival time. The estimated LC50 value was 2320 mg m^{-3} (1990 to 2730 mg m^{-3}, 95 percent confidence limits).

When Sprague-Dawley rats received five daily 4-hr exposures to 350 or 990 mg m^{-3} of RP/BR aerosol only one died. Comparison of body weights and clinical observations for the 1- and 4-hr studies showed no effects after the 1-hr and negligible ones after the 4-hr exposures. Exposure concentration was the dominant variable for induction of lethal effects.

(3) $RPNO_3$

There are no data available.

c. Repeated Inhalation Exposure Effects

(1) WP/Felt

There are no data available.

(2) RP/BR

For four consecutive days/week for 2.25 hours/day male and female rats were exposed for four weeks to 500, 1000, and 1200 mg m^{-3}, and 400, 750, and 1000 mg m^{-3} respectively. During the exposure period, wheezing and labored breathing were observed in male rats exposed to the high dose. Decreased body weights, body weight gains, and food consumption were seen in male rats at all exposure concentrations returned to normal after the recovery period. Although an overall mortality of 12.1% was observed in male rats exposed to the high dose, this was due to a 70-minute concentration overrun to 1650 mg m^{-3} in one of the chambers on the first day of the exposures. The 5.2 percent mortality observed in a second chamber reflects the effect of the exposure more realistically. In female rats only a single death was observed during the entire study and this mortality occurred in the middle concentration group (750 mg m^{-3}).[17]

Decreased WBC counts were noted in the male rats in the 750 and 1000 mg

m^{-3} groups at the end of exposure only. Increased blood lymphocytes were seen in the 750 mg m^{-3} female rats both at the end of exposure and after a recovery period. Decreased cholesterol and BUN values were seen in all RP/BR-exposed male rats, and concentration-related decreases in cholesterol, and triglyceride levels were seen in all RP/BR-treated females immediately after the exposure. After the recovery period, only female rats at 1000 mg m showed significantly decreased cholesterol and triglyceride levels, whereas female rats in the 750 or 1000 mg m^{-3} groups showed decreased BUN levels.[17]

There were significant increases for cellular ATP levels, expressed as ATP/cells or ATP/protein, tested immediately after the last exposure at all three concentrations (750, 1000 and 12030 mg m^{-3} for male and at the low and medium concentrations (400 and 750 mg m^{-3}) for female rats. After recovery ATP/protein from male rats exposed to the high dose remained elevated, whereas ATP/cells and ATP/protein both were increased in macrophages from female rats that had received the high dose (1000 mg m^{-3}). The most consistent finding in macrophages of male and female rats from all treatment groups was decreased activity of the plasma membrane-associated ectoenzyme 5'nucleotidase (5'ND). In addition, macrophages of male rats tested after a 14-day recovery also had decreased alkaline phosphatase (APD1) activities. Decreased activity of both 5'ND and APD1 in macrophages has been associated with enhanced in vitro antitumor and antiviral activity. These data suggest that there may be a change in alveolar macrophage population induced by exposure to RP/BR and that these cells may have been primed for activation or activated as evidenced by the significant increases in cellular ATP levels.[17]

A significant increase in the protein level of the pulmonary lavage fluid of rats of both sexes after exposures to the high doses (1200 and 1000 mg m^{-3} for males and females respectively) indicates pulmonary edema, which was resolved during the recovery period. Total cell counts were significantly increased in the pulmonary lavage from female rats immediately after exposure to 750 or 1000 mg m^{-3}, while differential counts remained unaffected indicating unaltered cellular distribution. After the 14-day recovery period the counts were no longer different from controls. Of the neurobehavioral parameters, locomotor activity was significantly affected by exposure to RP/BR aerosols. Male rats showed increased motor activity at all concentrations and incomplete recovery after two weeks at some concentrations. In females there was a trend toward increased activity but no evidence of effects after the recovery period. None of the other behavioral endpoints were reliably altered by the exposures.[17]

The respiratory tract was the main target organ for structural changes. The primary lesion was terminal bronchiolar fibrosis evident after exposure to 400 mg m^{-3} for 3.5 hours per day for four consecutive days. The lesion increased in incidence and severity with increasing concentrations and length of exposure and did not exhibit recovery during the 14-day holding interval after exposure. Masson's trichrome stain was used to confirm and grade the amount of collagen in the terminal bronchioles and

associated alveoli in the affected animals. The results indicate that part, but not all, of the thickening of the terminal bronchioles and associated alveoli was due to the formation of new collagen fibers. The severity of the lesion was concentration-related in both sexes, and was observed at all concentrations. There was a peribronchiolar and perivascular infiltration of eosinophils which regressed during the recovery period. Several of the animals exposed to the two higher dosage levels had a slight increase in inflammation of the posterior nasal turbinates relative to controls as shown by an increase in the number of lymphocytes in the submucosa with infiltration of the mucosa by the same cells.

(3) $RPNO_3$

There are no data available.

d. Subchronic Inhalation Exposure Effects

(1) WP/Felt

Sprague-Dawley rats were exposed for 15 minutes/day, 5 days/week for 13 weeks to 1000, 500, and 200 mg m^{-3}.[17] Exposure was followed by a 4-week recovery period. No visible toxic signs were observed at the low and middle concentrations. Animals appear to develop a tolerance, especially at the lowest dose. Animals were examined for exposure-related effects after six and thirteen weeks of exposure and after four weeks recovery following thirteen weeks of exposure.

After six weeks of exposure, all laryngeal and tracheal specimens from rats exposed to the highest concentration displayed moderate to severe laryngitis and tracheitis. Fifty percent of the rats exposed to the intermediate concentration displayed minimal to mild tracheitis while one-third had a mild laryngitis. Only one rat exposed to the lowest concentration displayed tracheitis. Four of six rats at the highest concentration had a minimal to severe interstitial pneumonia. Similar histopathologic lesions were present after the end of 13 weeks of exposure. After a 4 week recovery period, lesions were noted in the larynx and trachea of 15 of 16 high concentration rats and 20 of 24 intermediate concentration rats. Pulmonary lesions were noted in 11 of 16 high concentration and 6 of 24 rats in the intermediate concentration group. None of the control or low concentration animals exhibited significant lesions.

(2) RP/BR

Laboratory rats, mice, guinea pigs and rabbits were exposed to RP/BR aerosols at daily concentrations in the range of 22 to 168 mg m^{-3}. Exposures were for 8 min/day, 5 days/week for 12 weeks. The results of the study demonstrated no significant exposure-related effects.[11]

Sprague-Dawley rats were exposed for 2.25 hours/day, 4 consecutive days/week for 13 weeks to 300, 750, and 1200 mg m^{-3}, followed by an 8-week recovery period.[18] The most striking and consistent effects observed in this study were the presence of terminal bronchiolar fibrosis for all RP/BR concentrations after the last exposure as well as at the end of the recovery period and the highly significant depression in the pulmonary bactericidal activity measured immediately after exposures. The fibrotic changes were observed in animals exposed to 750 and 1200 mg m^{-3} two weeks after the start of the exposure. The fibrotic lesion was shown to contain increased amounts of collagen as well as calcium salts. Additional studies are being conducted at lower concentrations using the same dosage regimen.

(3) $RPNO_3$

There are no data available.

e. Reproductive/Teratogenic Effects

(1) WP/Felt

The results of a single-generation reproduction study, in which Sprague-Dawley rats were exposed to 589 and 1161 mg m^{-3}, were difficult to interpret because it was not possible to determine the cause of a deficiency in fetal weight gain at the 1161 mg m^{-3} exposure level. Pregnant Sprague Dawley rats were exposed from days 6 to 15 of gestation to 589 and 1161 mg/m^3. No teratogenic effects were observed.[17]

(2) RP/BR

No reproductive effects were observed in Sprague-Dawley rats exposed to 132 mg m^{-3} or 1186 mg m^{-3}.[11] No significant teratogenic effects were observed in Sprague-Dawley rats exposed to 132 mg m^{-3} or 1186 mg m^{-3} during the period of organogenesis.[11]

(2) $RPNO_3$

There are no data available on either reproductive or teratogenic effects.

g. Mutagenic Effects

(1) WP/Felt

Results of a sex-linked recessive lethal test in Drosophila, in which the test organism was exposed to WP/felt smoke aerosol condensate, were negative.[15]

Results of a dominant lethal mutation study performed on Sprague Dawley rats exposed to 1161 and 589 mg m^{-3} WP/felt smoke aerosol were negative.[17]

(2) RP/BR

No dominant lethal mutations were observed in Sprague-Dawley rats exposed to 132 or 1186 mg m^{-3} RP/BR smoke aerosol.[11] No sex-linked recessive lethal mutations were observed in Drosophila exposed to this smoke. The results of a micronucleus assay performed on the bone marrow of female Sprague-Dawley rats showed a positive effect after 2 weeks exposure to 1000 mg m^{-3}, but no effects after 4 weeks exposure or after a two-week recovery.[19]

The results of an Ames plate-incorporation test, an in vitro cytogenetics test, and an unscheduled DNA synthesis test showed no mutagenic effects of the RP/BR smoke aerosol condensate. In some tests, the low pH of the aerosol condensate produced false positive results.[18]

h. Carcinogenic Effects

There are no data available on the phosphorus smokes.

4. Summary

Phosphorus smoke aerosols act as irritants because of their high phosphoric acid content. Respiratory irritation has been observed in humans exposed to white phosphorus smoke. Respiratory irritation and inflammation have been noted in laboratory animals exposed to phosphorus smokes. In addition, recent studies on the effects of RP/BR smoke aerosol have shown terminal bronchiolar fibrosis and a decline in pulmonary bactericidal activity in laboratory rats exposed to the aerosol. The fibrotic changes appear to be irreversible. The induction of fibrotic changes appears to be influenced by both concentration and daily exposure duration. Concentrations of 300 mg m^{-3} for 2.25 hours elicited these effects whereas daily exposures of 8 minutes at 168 mg m did not produce histologic lesions. While no skin effects have been reported in the literature, skin irritation might be expected following exposure to phosphorus smoke condensates because of their high phosphoric acid content. No significant reproductive, teratogenic, mutagenic or carcinogenic effects have been reported for the phosphorus smokes. Because of the close similarity of the composition and characteristics of the phosphorus smokes, the RP/BR smoke aerosol is used as the model for toxicologic effects.

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E. Brass

1. The bulk material and disseminated smoke from the M76 grenade have the same chemical and physical characteristics. The brass is an irregular flake which has a diameter of 1.7 microns and a thickness between 0.08 to 0.32 microns. The flakes are coated with palmitic and/or stearic acid.[1] Analyses of metal contaminants in five separate lots of this material are reported in Table 1. The Mass Median Aerodynamic Diameter (MMAD) is 2.1 to 2.3 um with σ_g of 1.6. Brass powder used as fill material for the M76 smoke grenades is being considered for other smoke applications. The unique characteristics (nonwetability) and delivery ease of this material make it an interesting subject when considering its environmental fate and effects.[1]

2. Pharmacokinetics/Metabolism

Analysis of bronchopulmonary lavage fluid, pulmonary function, and histopathological evaluations were used as biological endpoints to compare the inhalation hazards of EA 5763, EA 5753D (Dedusted), and EA 5752. Groups of male Fischer 344 rats were exposed four hours to one of five dosage levels of either EA 5763, EA 5763D (200, 100, 50, 10, 1 mg/cu m) or EA 5752 (1000, 200, 100, 50, 10 mg/cu m). At 24 hours, 14 days, and three months post exposure, the rats were evaluated for physiological and histological alterations to correlate with enzymatic and cytological profiles of lavage fluid. At 24 hours post exposure, in rats exposed to EA 5763 and EA 5763D, there were dose-related changes at all but the lowest concentration (1 mg/cu m). In rats exposed to EA 5763 and EA 5763D, there were increases in lactate dehydrogenase and protein in the lavage fluid, increased pulmonary resistance, acute inflammatory response in terminal airways, and increases in macrophages and neutrophils. All reactions were resolved by 14 days post exposure. In contrast, EA 5752 produced no alteration in pulmonary function, but elicited persistent changes in the enzymatic and cytological parameters of the lavage fluid, with multifocal microgranulomas in lung and hilar lymph nodes. Respiratory protection is recommended when personnel are exposed to airborne EA 5752, EA 5763, and EA 5763D.[2]

3. Health Effects

a. Skin and Eye Irritation

The application of 80 mg of brass to abraded and unabraded skin of six rabbits was nonirritating for five of 6 rabbits. One rabbit showed mild edema on the abraded site and slight edema on the unabraded site at 24 hours. All signs were resolved by 72 hours. This material was considered to be practically nonirritating to rabbit skin.[3]

In the eye irritation test, 80 mg was instilled into the inverted lower side of the right eye of three rabbits. The eyes were exposed for 24 hours. Slight to mild corneal opacity was observed in two eyes at 24 hours and in the other eye by day 7. Only one eye displayed iritis at 24 hours which resolved by the 72 hour observation period.

Hyperemia, chemosis, and discharge were observed in all three treated eyes at 24 hours post application. Hyperemia resolved in all eyes by day 7; chemosis was resolved by 72 hours, and no discharge was observed by 48 hours. This material is mildly irritating to rabbit eyes when exposure lasts for 24 hours.[3]

In another study, brass powder was found to be a mild skin irritant (calculated primary irritation index was 2.0); tested positive for eye irritation; had a dermal LD 50 greater than 2 g kg^{-1}; and an oral LD50 of 1586.9 mg/kg in males, 1696.1 mg kg^{-1} in females and 1561.2 mg kg^{-1} for the combined sexes. Histopathologic examination of test and untreated skin sites from two males and two females used in the dermal toxicity study revealed treated skin from one male had mild dermal edema and fragmentation of collagen and the other male and minimal hyperkeratosis and focal keratotic entrapment of the test material and debris. All the untreated skin specimens and treated skin from both female rabbits were judged to be unremarkable.[3]

b. Acute Oral Toxicity

Brass given by intragastric intubation to male and female Fischer 344 rats at 5000 and 3500 mg kg^{-1} produced mortality in most of the animals. A dose of 3300 mg kg^{-1} was lethal to the five female rats treated, but none of the five male rats. The toxic signs in both sexes were loss of body weight, diarrhea, lethargy, hunched posture, piloerection and epistaxis. Two of the five female rats receiving the material at a dose of 1980 mg kg^{-1} died by day 7, none of the five male rats died. Toxic signs were similar to those observed after doses of 315 mg kg^{-1}. The oral LD50 for male rats is less than 3500 but greater than 3300 mg kg^{-1}. The LD50 for the female rat is 2054 mg/kg with 95 percent confidence limits between 1623 and 2676 mg kg^{-1}.

The aquatic toxicity of a brass particulate was also examined. Acute, 48 hour bioassays were performed using the water flea, Daphnia magna. Tests were conducted with uniform suspensions of uncoated brass particulate, brass particulate coated with a tetrafluoroethylene solution, silica, and titanium dioxide. The Teflon coating solution and the supernatant for the brass suspension (after settling of the brass) also were tested. All tests were conducted according to guidelines set forth by the US Environmental Protection Agency (EPA) and the Organization for Economic Corporation & Development (OECD). The effective concentrations that could be lethal to 50% of a population were calculated for uncoated (20.9 ug L^{-1}) and coated (3.6 ug L^{-1}) brass particulate. The silica, titanium dioxide, and Teflon each had an EC50 of greater than 1 g L^{-1}. Chemical fate studies demonstrated that the brass dissociated to its ionic components of copper and zinc quickly to pH 2.0. At pH 5.0 and 6.5, the dissociation occurred too slowly to hypothesize that the observed toxicity is due to filtration by the daphnids and subsequent ingestion. Ingestion of particulates causes acute effects due to determinations for the brass are nearly identical with published EC50 values for copper salts.[4]

c. Acute Inhalation Effects

Groups of male Fischer 344 rats were exposed for four hours to brass concentrations of 1, 10, 50, 100, and 200 mg m^{-3}. At 24 hours post-exposure, there were dose-related increases in lactate dehydrogenase and protein in lung lavage fluid, increased pulmonary resistance, acute inflammatory response in the terminal airways and increases in numbers of macrophages and neutrophils. All toxic signs were resolved by 14 days post-exposure.[5]

Lung leukocytes collected by bronchopulmonary lavage from control rats were more than 90 percent macrophages. In rats exposed to the first three days after exposure suggesting an inflammatory response. The macrophages (16 percent) during this period were either bi- or multi-nucleated. This pattern of increased nucleation persisted for two weeks after exposure. Increased macrophage phagocytic activity remained elevated for 9 days, whereas chemotaxis was initially inhibited bud was considerably enhanced 7 to 14 days post-exposure.[6]

Pulmonary alveolar macrophages (PAM) actively protect thelungs from infection by ingesting particles of all kinds that reach the alveoli. To expand our data base onthe physicological effects of inhaled particlulates, the in vitro cytotoxicity of EA 5752, EA 5755, EA 5763, and EA 5763D in the Pulmonary Alveolar Macrophages (PAM) of the rat was determined. Trypan blue exclusion was used as the criterion of viability. The four hour LC50s for EA 5752 and EA 5755 were 72.6 ug mL^{-1} and 35.5 ug mL^{-1}, respectively. The dose response curves for EA 5763 and EA 5763D were complex, precluding exact LC50 determinations; however, both agents were cytotoxic at concentrations 1-3 ug mL^{-1}. None of these particles were phagocytized in vitro, but in all cases numerous particles were attached to PAM membranes. Particle-free supernatants for EA 5752 and EA 5755 had little effect on PAM viability. Extracellular serum proteins protect PAM from the cytotoxic effects of EA 5763 and EA 5763D.[7]

This study was performed to permit the eventual differentiation between expressions of direct cytotoxicity and the more subtle physiological changes induced by agent exposure.

d. Repeated Inhalation Exposure Effects

The toxic effects of repeated exposures to brass, including efects of concentration, exposure duration and frequency of exposure are under investigation.

e. Teratogencity/Reproductive

The effects of brass flakes, as a potential teratogen, in producing a dominant lethal mutation (DLM) or on reproduction in two generations (TGs) of rats were investigated. There were no significant effects inany of the studies conducted at the 15 min/day expsure period. At the 150 min/day period, however, the wastage of the rats was severe and most of the rats, witht the exception of the teratology dams, died during the exposures. The wastage and deaths complicated the detection of any

effects of the brass on measured parameters in all studies.[8]

4. Summary

Short-term inhalation of brass flakes by rats in particle size range of 2.1 to 2.3 um Mass Median Aerodynamic Diameter (MMAD) produces reversible toxicity ans manifested by increased enzymatic activity in bronchopulmonary lavage fluid, influx of inflammatory cells and histopathologic changes. The effects of repeated inhalation of this material are under investigation.

The material does not produce significant skin irritation, and eye irritation after a 24 hour treatment is mild and reversible.

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F. Dyes in Inventory, Product-improved, and Developmental Colored Smokes

1. General Information and Properties

a. Bulk Material.

(1) Product Improvement Program. The colored smoke mixes are incorporated into the M18 smoke grenade along with a pyrotechnic mixture. The component dyes in the yellow smoke grenade have been replaced with a totally new formulation. The yellow component of the green smoke grenade has also been replaced. These two product-improved grenades have been type-classified. A product improvement program is under way to replace the dye components of the red and violet grenade with less biologically active materials. As of September, 1993, the red and violet dye mixtures incorporated into the M18 grenade remain the ones identified as the "old" mixes. The 40mm cartridge grenade is still made using the "old" mixtures. Because grenades with the older formulations remain in the system, the information is presented on the compositions and toxicity of both old and product-improved grenade formulations and the smoke they produce upon being functioned.

(2) Old Inventory Colored Smoke Grenades. In the "old" M18 grenades, the colored smoke dyes are mixed with sulfur, potassium chlorate, and sodium bicarbonate, with optional amounts of water-white kerosene and tricalcium phosphate for control of dusting and caking, respectively. The dye components of the old yellow grenade (B143-4-1) are Dye. Yellow MIL-D-0050029 (1,4-diamino-2,3-dihydroanthraquinone) (DBC) and benzanthrone. The dyes in the old green smoke grenade (B143-2-1) are those in the old yellow grenade plus Dye, Solvent Green, MIL-D-003277(1,4-di-p-toluidino-9,10-anthraquinone)(PTA). The red smoke grenade contains Dye, Red Disperse 9, MIL-D-3284 (l-methylaminoanthraquinone) (MAA). The old violet smoke grenade contains Disperse Red 9 plus Dye, Violet, MIL-D-3668 (1,4 diamino-2,3dihydroanthraquinone) (DDA).

The old dye mixtures have been studied extensively by Rubin and Buchanan[1]. The mixes were fractionated by vacuum sublimation, differential solubility, and liquid chromatography. The major components were isolated and identified by comparison with the pure dyes using a variety of instrumental techniques. A number of contaminants at very minor levels were identified by gas chromatography/mass spectroscopy. All of the smoke mixes contained relatively large quantities (10-25 percent) of chloroform-insoluble or non-volatile undifferentiated carbonaceous material. The identified components of the four old smoke mixes are outlined below.

Old yellow smoke mix

Major components: Dibenzochrysenedione (DBC), benzanthrone (BZA)
Insoluble residue: 22%
Minor components (< 1 %): unidentified diketone MW = 366.

Old green smoke mix

Major components: 1 ,4-di-p-toluidino-9, 10-anthraquinone (PTA), benzanthrone (BZA), and dibenzochrysenedione (DBC)
Insoluble residue: 9 %
Minor components (< 1 %): high molecular weight, none identified.

Old red smoke mix

Major component: l-methylaminoanthraquinone (MAA)
Insoluble residue: 14 %
Major contaminant: anthraquinone (2 %)
Minor contaminants (~1 %): aminoanthraquinone, 2-methylamino anthraquinone.

Old violet smoke mix

Major components: 1,4-diamino-2,3-dihydroanthraquinone (DDA) and 1-methylaminoanthraquinone (MAA)
Insoluble residue: 24%
Minor contaminants (< 1 %): anthraquinone, aminonapthalene, arninoanthraquinone, phenyldiazobenzene.

(3) Product Improved Colored Smoke Grenades. In the new and developmental colored smoke grenades designed to replace those containing the old dye mixes, sugar has replaced sulfur as the fuel, and magnesium carbonate is used as a coolant instead of sodium bicarbonate. The developmental red grenade also contains terephthalic acid and stearic acid in addition to the dye and pyrotechnic ingredients. The type-classified new yellow and green colored smoke grenades have been reformulated to replace the benzanthrone and DBC with a single component: Dye, Solvent Yellow 33, DOD-D-51485A(EA) [2-(2'-quinolyl)-1,3-indandione] (QID). The PTA is retained in the product-improved green grenade, combined with QID instead of benzanthrone and DBC. Thus some of the results of chemical characterization of the deployed smoke from functioned green smoke grenades and those portions of the toxicity information developed using the PTA fraction of the green smoke mixture are applicable to the product-improved green smoke grenade as well.

Three new dyes have been identified for use in the developmental red and violet grenades designed to replace those containing the old dye mixes, sugar has replaced sulfur as the fuel, and magnesium carbonate is used as a coolant instead of sodium bicarbonate. The developmental red grenade also contains terephthalic acid and stearic acid in addition to the dye and pyrotechnic ingredients. The type-classified new yellow and green colored smoke grenades have been reformulated to replace the benzanthrone and DBC with a single component: Dye, Solvent Yellow 33, DOD-D-51485A(EA) [2-(2'quinolyl)-1,3indandione] (QID). The PTA is retained in the product-improved green grenade, combined with QID instead of benzanthrone and

DBC. Thus some of the results of chemical characterization of the deployed smoke from functioned green smoke grenades and those portions of the toxicity information developed using the PTA fraction of the green smoke mixture are applicable to the product-improved green smoke grenade as well.

Three new dyes have been identified for use in the developmental red and violet grenades, completely replacing the MAA and DDA previously used. The developmental red smoke grenade contains Dye, Disperse REd 11, DOD-D-514522(EA) (a-methoxybenzenazo-1-napthol) (MBN). A developmental violet smoke dye mix consisted of DMA and Dye, Disperse Blue 3, DOD-D-51524, {1-[(2-hydroxyethyl)-amino]-4-(methylamino)-9, 10-anthraquinone} (HEMA). This violet smoke formulation has been abandoned because of toxicity information developed during the testing of this dye mixture.

The compositions of the dye components of the product-improved M18 grenades have been studied by Buchanan and Ma[2], and the dye components are as follows.

Product-improved yellow smoke mix

Major component: 2-(2'-quinolyl)-1,3-indandione (QID)
Insoluble residue: 3.7 %
Minor contaminants: none detected.

Product-improved green smoke mix

Major components: 1,4-di-p-toluidinoanthraquinone (PTA) and 1-(2'-quinolyl) 1,3-indandione (QID)
Insoluble residue: 0.6 %
Minor contaminant (<1%): 1-p-toluidinoanthraquinone (TA)

Developmental red smoke mix

Major components: a-methoxybenzenazo-b-napthol(MBN)and I,4-diamino-2methoxyanthraquinone (DMA)
Insoluble residue: 1.6 %
Minor contaminant (< 1 %) aminoanthraquinone.

Developmental violet smoke mix (abandoned)

Major components: 1,4-diamino-2-methoxyanthraquinone (DMA), 1-(2-hydroxyethylamino)-4-(methylamino)-anthraquinone (HEMA), 1,4-bis(methylamino)anthraquinone, and 1,4-bis(2-hydroxyethylamino)anthraquinone
Insoluble residue: 3.2 %
Minor contaminants: none detected.

b. Combustion products.

In separate studies, Rubin and Buchanan[1] and Buchanan and Ma[2] characterized the components of the smoke produced by the old red and violet grenades and all four colors of the new M18 grenades. The grenades were functioned inside canvas tents and samples were collected of the vapors and particulates generated by the grenade. Chin et al[3]. studied the combustion of U.S. Navy red, yellow, and green smokes which use the same dye constituents as the Army smokes. In this study the grenade fill, including the dyes, the fuel, the oxidizer, the cooling agent and binders, was thermally vaporized, and the solids and vapors produced were collected on filters and traps, respectively and analyzed by thin layer chromatography, high performance liquid chromatography, gas chromatography, gc-mass spectrometry, nuclear magnetic resonance, ultraviolet spectrometry, and electron dispersive x-ray analysis. The results of both studies were in agreement in the case of the red smoke grenade fill, the only case of overlap in the two studies. The changes effected by detonation of the red and violet grenades are listed below. There were no major chemical changes noted by Chin and his coworkers in the compositions of the old yellow and green smoke grenades when they were sublimed at 400600°C and re-condensed into ambient air.

Old green dye mix (Reference 3)

No major changes noted.

Old red dye mix (Reference 1)

Major component (MAA) converted (12 %) to other compounds, chiefly 1- and 2 aminoanthraquinones (1-AA and 2-AA).
Particle diameters: 2.3 um (7 min.); 2.8 um (26 min.) [Times given in parentheses are post-ignition.]

Old violet dye mix (Reference 1)

Major component (DDA) converted (100 %) to 1,4-diaminoanthraquinone.
Minor component (MAA) partially converted to 1- and 2-AA.
Particle diameters: 1.1um (2 min.); 2.3 um (26 min.)

Product-improved yellow dye mix (Reference 2)

Approximately 5% of QID was converted to oxidized products, tentatively identified as quinolylnapthalone and isomers of quinolylnapthoquinone.
Insoluble residue: 5.0 - 9.9 %
Particle diameters: 0.85 um (3 min.); 1.80 um (30 min.)

Product-improved green dye mix (Reference 2)

A small fraction of PIA was altered when the grenade was functioned, while QID remained relatively unchanged. New products formed (totaling about 3 % of original dye weight) were tentatively identified as l-p-toluidinoanthraquinone and an isomer of QID.
Insoluble residue: 3.4 %
Particle diarneters: 1.57 um (3 min.); 2.05 um (30 min.)

Developmental red dye mix (Reference 2)

Major dye components, MBN and DNA, were not affected to a great extent by functioning of the grenade. There were no new components formed that were in excess of 1 % of the recovered particulates. Minor components (<1 %) that have been tentatively identified include: methoxyaniline and an alkylmethoxyaniline, napthols, cyanonapthols (?), phthalate esters, and acetyl anthraquinone.
Insoluble residue: 3 .7 - 4.5 %
Particle diameters: 2.33 um (3 min.); 3.32 um (30 min.)

Developmental viclet dye mix (Reference 2)

One of the components of the Disperse Blue 3, 1,4-bis(2-hydroxy-ethylamino)anthraquinone, was not detected in the collection smoke particulates and can be assumed to have undergone chemical change in the process of functioning the grenade. Components found in the smoke, range from 1-3% of the particulate mass, and not present in the smoke mix were: 1-aminoanthraquinone, 1,4-diaminoanthraquinone, and (tentatively identified): aminomethoxyanthraquinone, 1-amino-4-methylaminoanthraquinone, methyl-substituted DMB, and dimethyl-substituted DMB.
Insoluble residue: 3 4%
Particle diameters: 1.4 um (3 min.); 2.7 um (30 min.)

2. Pharmacokinetics/Metabolism

Henderson et al.[4] studied the toxicity of inhaled aerosols of the dye formulations for the product-improved yellow and green M18 grenade. After 90 day exposures to the yellow smoke dye (QID) the concentrations in the lungs ranged from 0.025 ug/lung for female rats exposed to 1 mg/m^3 to 1.3 ug/lung for males and females exposed to 100 mg/m^3. For 90-day exposures to the green smoke dye mixture (PTA/QID), the retention of PTA was much greater, ranging from 39 ug/lung in female rats exposed to 1 mg m^{-3} to 2800 ug/lung in females and 3400 ug/lung in males exposed to 100 mg/m^3 . According to Henderson et al., approximately 19 % of the calculated amount of PTA that would have deposited in lungs during the 90-day

exposures was found in lungs at every exposure level. (QID) was not detected in any of the groups exposed to PTA/QID.) In addition, 8 other groups of 3 male and 3 female rats were killed from 3-230 days after the last day of exposure and lungs were analyzed for green smoke mixture components. Even after 230 days, the high-exposure rats contained 2100 ug PTA/lung (female) and 3600 ug PTA/lung (male), indicating a very low clearance rate for PTA.. In a tracer study using a mixture of QID and ^{14}C-PTA, the QID was rapidly cleared from the lungs and was extensively metabolized prior to excretion, while the PTA was retained in the lungs. The disposition of QID did not appear to be affected by the presence of PTA.

There are no metabolism or toxicokinetics studies of the old red and violet mixtures. In 4- and 13-week inhalation studies of the developmental red dye mixture conducted by Costa et al.[5], all of the deposited dye within the lung had been cleared within two weeks post exposure. At the end of 13 weeks exposure to the developmental red dye mixture, pigmented granules could be discerned in the kidney tubules in a dose-dependent manner, slightly more in the females. This effect was not apparent in animals that had been allowed to recover for 4 weeks post exposure. The violet dye mixture, after single exosures, was found throughout the carcasses of expired and sacrificed animals.

3. Health effects

a. Skin and eye irritation

1-Methylaminoanthraquinone, an ingredient in the old red smoke mix, is reported as a skin irritant and sensitizer in humans[6], although another source cited in the same document[6] states that there was no skin or eye irritation for rabbits. 2-Aminoanthraquinone, identified as a major product in the smoke from the old red grenade and the developmental violet grenade, caused severe eye irritation, at a dose of 100 mg for 24 hours, in rabbits[7]. Benzanthrone, an ingredient of the old yellow smoke mix, is reported to cause an itching, burning sensation, erythema, dermatitis, and skin pigmentation in humans. The dye was reported as moderately irritating to the skin of rabbits (500 mg/24 hours) and produced severe eye irritaion in rabbits (100 mg/24 hours)7. 1,4-PTA, present in both the old and the product-improved green smoke mixes, produced a severe reaction in 24 hours at a dose level of 20 mg in rabbit eyes, according to one study[8], but in another study[9], Muni and coworkers found the mixture of PTA and QID which makes up the product-improved green dye mixture to be non-irritating in the rabbit primary eye irritation assay. Muni et al. found QID to be "practically non-irritating" in the primary eye irritation assay. QID, the dye component of the product-improved yellow M18 grenade, and the mixture of QID and PTA were found to be "practically non-irritating" in the rabbit primary dermal irritation study as well.[9] 1,4-Diaminoanthraquinone, the compound to which 1,4-diamino-2,3-dihydroxyanthraquinone, a major ingredient in the old violet dye mix, is almost

quantitatively converted when the grenade is functioned, has been found to be a moderate eye irritant in rabbits at a dosage of 500 mg for 24 hours.[7] Costa[5] conducted a variety of immunotoxicology studies to assess the potential allergencity of the develomental red dye mixture. In an inhalation test in guinea pigs the new red mixture showed no potential for respiratory sensitization. In a mouse ear sensitivity test, modified by supplementing the diet with Vitamin A, the Solvent Red 1 component was found to be a mild sensitizer, in the class of formaldehyde, which was used as the positive control. The Disperse Red 11 component was negative in this assay. There are no irritation data on the major ingredients and products of combustion not listed.

b. Lethal doses

Smoke mix	Compound	Species	Route	LD50	Ref.
Old red	1-methylaminoanthraquinone				no data.
	1-aminoanthraquinone	rat	i-p	I.5g/kg	7
Old	1,4 diamino-2,3 dihydroanthraquinone				no data
Violet	1,4-diaminoanthraquinonerato	rat		4.9 g/kg	7
Old yellow	dibenzochrysenedione (DBC)	rat	derm	>4.6g/kg	7
	benzanthrone	rat	i-p	1.5 g/kg	7
	mousei-p			0.29 g/kg	7
Old green	DBC and benzanthrone (see above) 1,4-di-p-toluidinoanthraquinone	rat	oral	3.1g/kg	7
New red	1,4diamino-2-methoxyanthraquinone	rat(f)	oral	>5 g/kg	21
		rat(m)	oral	7-1.0 g/kg	21
	a-mehoxybenzenazo-b-napthol			>5 g/kg	21
	Mixture			>5 g/kg	21
New violet	1,4- diamino-2,3-dihydroxyanthra-quinone	rat	oral	3 g/k	7

Smoke mix	Compound	Species	Route	LD50	Ref.
New yellow	2-(2'-quinolinyl)-1,3-indandione (QID)	rat	oral	>5 g/kg	8
New green	QID (see above)				
	1,4-di-p-toludinoanthraquinone (PTA)	rat	oral	3.1 g/kg	7
	Mixture (PTA/QID)	rat	oral	>5 g/kg	8

c. Inhalation exposure effects

i. New yellow and green smoke mixes. A three-phase inhalation toxicity study of the product-improved yellow and green smoke dye mixtures was carried out by Henderson et al. In the acute study[10], three male and three female Fischer 344 rats per group were exposed to concentrations of QID of 1000 (lhr), 1040 (6 hr), and 1290 (6 hr for 5 days) mg/m^3. The respective concentrations of the mixture of PTA and QID were 1600, 1440, and 1490 mg/m^3 for the same exposure regimes. There were no overt signs of toxicity after any of the exposure regimens. Histologic examination of the respiratory tract from rats exposed to both test materials for 5 days showed goblet cell hypertrophy and hyperplasia of the respiratory epithelium of the nasal cavity. In this area, there was a mild serous inflammation of the respiratory epithelium and degeneration of the olfactory portion. In the lung parenchyma, there were a few focal accumulations of alveolar macrophages centered on terminal airways. Pigment-laden macrophages were found in the medulla of the tracheobronchial lymph node.

In four-week studies[11], rats were exposed for 6 hours per day, 5 days per week, to both test materials. The QID concentrations were 10, 51, and 234 mg/m^3, with a MMAD of 3.1 to 4.1 um and a σ_g of 2.0. Animals exposed to the highest QID concentration had a reduced body weight gain (8 percent less than controls). Mild respiratory function changes consisting of reduced elastic recoil, increased resting lung volumes and reduced expiratory flow were found at the highest concentration. Histopathologic lesions were not observed. For the QID/PTA mixture, rats were exposed to 11, 48, and 208 mg/m^3 with MMAD of 3.1 to 4.7 um and a σ_g of 2.0. Reduced weight gain was observed at the highest concentration. Respiratory function tests on the high concentration group showed a reduction of gas exchange efficiency and airflow obstruction. Lung lavage fluid analyzed showed a mild inflammatory response to the highest concentration. Respiratory function tests on the high concentration group showed a reduction of gas exchange efficiency and airflow obstruction. Lung lavage fluid analyses showed a mild inflammatory response to the highest concentration. Histopathologic lesions consisted of Type II cell hyperplasia and proliferation of "foamy" alveolar macrophages.

In the subchronic inhalation study, rats were exposed to concentrations of each aerosol of 0, I, 10, and 100 mg/m^3 with particle MMAD of 2.1-4.2 um and σ_g of 2.0. Animals exposed to the 100 mg/m^3 of QID had only a slight decrease in body weight gain (4 percent compared to controls) and an accumulation of foamy macrophages in lungs. Exposure to the lower concentrations of QID elicited no observed response. Animals exposed to IO0 mg/m^3 QID/PTA had pulmonary inflammation (indicated by increased lactate dehydrogenase activity, protein, and neutrophil numbers in lung lavage fluid) with histopathological evidence of mild Type II pulmonary epithelial cell Hyperplasia and proliferation of foamy alveolar macrophages. Some of the animals exposed to 10 mg/m^3 f QID/PTA produced similar, but milder, histopathological results. Thus, the no-observable-adverse effects level for inhalation exposure to QID from the product-improved yellow M18 grenade was 10 mg/m^3 while that of the mixture of QID and PTA in the product-improved green M18 grenade was 1 mg/m^3 under the exposure regime used.

ii. Developmental red and violet smoke mixes. A three-phase inhalation toxicity study of the developmental dye mixes for product-improved M18 grenades was completed in 1991 by Costa et al. at the Health Effects Research. Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC. The protocols were similar to those used by Henderson et al for the yellow and green dyes, and Fischer 344 rats were used in this study as well. The acute phase of the study was completed for the red dyes without producing any signs of toxicity from single six-hour exposures to concentrations of 100, 300, and 1000 mg/m^3 of the red dye mixture. Pathologic examination of the animals after these exposures revealed treatment-related lesions in the nasal cavities and lungs of male and female rats. The lesions consisted of a transient infiltration of neutrophils into the mucosa of the anterior nasal septum and secondary and tertiary bronchioles of the lung on the day of exposure. These were minimal lesions, according to the pathologist, they were unassociated with degenerative changes, and they had resolved by the third day post exposure. Three days after exposure there was also minimal to mild hyperplasia of mucous cells in the mucosa of the anterior nasal septum. This change had resolved in all but 2 of 22 animals by the seventh day after exposure. The amount of red dye mixture deposited in the lungs at the maximum exposure of 1000 mg/m^3 ranged from a maximum of 438 ug per gram of lung on the day of exposure to 0.96 ug/g in a rat examined 7 days post exposure. Thirteen-week exposures, 6 hours per day, 5 days per week to concentra~ons of 30, 100, and 300 mg/m3 of the red dye mixture. produced a subtle reStnCtiYe lung disorder that was par~ally to completely reverse: during a 4-week recovery period. There was no significant lung pathology, and the lung burden of the dye mixture was below detechble limits by 3 days postexposure. Histopathology from the 4-week study indicated that there were slight changes in the livers and spleens of the exposed rats, most apparent in the females. The males had effects limited to the nasal cavity. However, all changes were considered minimal to slight and not biologically significant. None of these histopathological changes were seen in animals

after 2 weeks in clean air. In the 13-week study, body weights were suppressed, the females being more adversely affected than the males. Exccept in the lowest exposure group (30 mg m^{-3}), males did not fully regain lost weight during the 4-week recovery period. Spleen changes consisting of a slight increase in hematopoietic cells were seen in the males preferentially at 13 weeks. This suggests reduced half-life of red blood cells (hemolytic anemia). This effect was reversible.

Single six-hour exposures of Fischer 344 rats to the violet dye mixture[13] resulted in 100 percent mortality at a concentration of 1000 mg/m^3, and half of the rats similarly exposed to 300 mg/m^3 were dead by three days post exposure. The remainder were all moribund and were killed at that time. Rats exposed to 100 mglm^3 survived through the three and seven day holding periods. Rats were exposed on five consecutive days, six hours per day, to 40 mg/m^3 of the violet dye mixture. Two days after the fifth exposure, 50 percent of the rats had died. Exposure-related lesions were noted in the nasal cavities and livers of male and female rats exposed to 100 mg/m^3 of the developmental violet dye mixture. The liver lesions were severe in males and resulted in several early deaths. In the females the lesions were mild to moderate, and in one animal sacnficed in moribund condition two days postexposure, the liver was histologically normal. The nasal lesion consisted of degeneration and necrosis of the olfactory epithelium and was equally severe in males and females. In the animals that survived the seven day postexposure period, the livers were normal. Since resolution of the lesions seen in the animals studied at Day 3 was unlikely to have occurred by Day 7, the pathologist concluded that there had been limited liver damage in these rats. The nasal lesions were severe in the animals examined at day three and in the surviving females. Early resolution was observed in the survivors. No effects of the exposure were seen in other organs. Lung burdens were similar to those for the red dye mixture, but there were no survivors at 7 days for the higher doses. The maximum deposition on the day of exposure was 221 ug/g in a rat exposed to 300 mg/m^3.

In order to further investigate the effects of the developmental dye mixture on the liver, rats were exposed by gavage to 800 mg/kg of each of the components of mixture, Disperse Blue 3 (DB3) and Disperse Red 11 (DR11) as well as to the complete violet dye mixture (VDM). The DR11 caused minimal changes in enzyme concentrations: DB3 caused significant increases (3 to 6 times control); and the VDM caused substantial increases in enzyme levels: 50 times control for serum glutamic-pyruvic transaminase (SGPT) These results suggest that a synergistic interaction of the two components of the VDM elicits toxicity via a rapid increase in hepatocyte metabolism, producing metabolites that are hepatotoxic.

d. Reproductive/Teratogenic Effects. There are no available data.

e. Mutagenic Effects

The following table summarizes the results of a study of the mutagenic effects of the old inventory dye mixtures and some of their components[14].

Smoke mix	Compound Assay	Results
Red	Whole mixture Ames	Positive
	MAA Ames	Positive
	Insoluble residue Ames	Positive
	1-Aminoanthraquinone DNA Damage	Positive
Violet	Whole mixture Ames	Positive
	1,4-Diamino-2,3 Ames -dihydroanthraquinone	Positive
	1,4-Diamino- Ames anthraquinone	Positive
Yellow	Whole mixture Ames	Positive
	Dibenzochrysenedione Ames	Positive
	Benzanthrone Ames	Positive
Green	Whole mixture Ames	Positive
	1,4-di-p-toluidino- Ames anthraquinone	Negative

Smoke	Command	Assay[a]	Results[b]	References[c]
Red	Mixture I[c]	Ames	Negative	12
	Mixture 2	Ames	Negative	12
	Terephthalic acid	Ames	Negative	12
	SolventRed 1 (MBN)	Ames	+I- w/S-9	12
			Pos w/S-9	14
		TK locus	No test	
Red/Violet	Disperse Red 11 (DMA) (DMA) Lot 1c	Ames	Negative	12
	Disperse Red 11 (DMA) Lot 2	Ames	Pos w/S-9	12
	Disperse Red 11	Ames	Negative	14
		TK locus	Pos w/S-9	14
Violet	Disperse Blue 3	Ames	Pos w/S-9	14
		TK locus	Positive	14
Yellow	Solvent Yellow 33 (QID)	Ames	Positive	13
		SCE	Negative	13
		TK locus	Positive	13
Green	Mixture (QID/PIA)	Ames	Positive	13
		SCE	Negative	13
		TK locus	Positive	13
	Solvent Green 3 (PTA)	Ames	Positive	13
		TK locus	Positive	13

NOTES:

a. Ames = Ames Salmonella assay; TK locus = L5178y/TK$^{+/-}$ mouse lymphoma assay; SCE = sister chromatid exchange in vivo in mice.

b. Negative indicates no significant mutations in all strains, with and without activation (if appropriate); Positive indicates that the compound produced a significant number of mutations in at least one strain without requiring activation; Pos w/S-9 indicated that the Ames Salmonella assay was negative or marginally positive without activation and was positive in at least one strain when activated with S-9 liver extract.

c. There were two lots of Disperse Red 11 (1,4-diamino-2-methoxyanthraquinone) tested by Henderson et al.[14], and the results in the Ames Salmonella assay were different, indicating that an impurity in Lot No. 2 may have been the active mutagen. Henderson et al.[15] conducted tests to determine the genotoxic potency of the red dye components of the developmental red and violet M18 grenade. Additional mutagenic

screening was performed by Moore et al.[16] on the new yellow dye, QID and the green dye, PTA, alone and in combination, and on the developmental red and violet dyes for the M18 grenade[17]. The above table summarizes those studies of the product-improved and developmental mixtures.

f. Carcinogenic Effects

Dibenzochrysenedione, an ingredient of the old yellow and green smoke mixes, has been tested in the NCI Bioassay Program and found to be a positive carcinogen in the male B6C3F1 mouse and negative in the female B6C3F1 mouse and in male and female Fischer rats (NCITR NCI-CG-TR-134,79, cited in References 6 and 7). The old red and violet smoke mixes were tested as complete carcinogens and as tumor initiators in the SENCAR mouse skin tumorigenesis bioassay system with negative results[18].

2-Aminoanthraquinone, one of the products formed by oxidative demethylation of 1-methylaminoanthraquinone in the deployment of red smoke from the old red smoke grenade, has been tested in the NCI Bioassay Program and found to be positive in both rats and mice (NCITR-NCI-CG-TR-144, cited in References 6 and 7) and is also classed as a suspected animal carcinogen by the International Agency for Research on Cancer[7]. 1-Aminoanthraquinone, the other product of demethylation of MAA is rated as an equivocal tumorigenic agent[7].

The Product-improved green dye mixture (QID/PTA) did not exhibit carcinogenic activity in the Strain A mouse lung tumor bioassay[19].

The developmental red and violet dye mixtures were tested in the rat tracheal epithelial cell focus assay[20] which measures the potential of the dyes to induce neoplastic transformation in respiratory tract epithelial cells. The red mixture (DMA/MBN) was positive in two assays. The violet mixture (DMA/HEMA) was positive in one assay, but was negative in a repeat test, and therefore the results are equivocal.

g. Other Observations

One member of the technical staff of the contractor investigating the toxicity of product-improved yellow and green dyes developed a skin rash after 5 days of working with the yellow/green mixture. Subsequent patch testing indicated that this individual was allergic to the yellow dye, but only weakly allergic to the QID/PTA mixture. The individual was also more allergic to the stock yellow dye than to the purified dye, suggesting that an impurity might be a potent allergen.

4. Summary

The dyes in the old colored smoke mixes were positive in the Ames Salmonella reversion assay. In addition, dyes in the yellow mix and products formed in the functioning of the old red smoke grenade have been shown to produce increased tumor incidences in rodent carcinogenicity bioassays. The major component of the old violet grenade is converted almost quantitatively to another compound which is positive in the bacterial mutagenicity assay.

The dyes in the product-improved yellow and green M18 grenade, Solvent Yellow 33 (QID) and the mixture of QID and Solvent Green 3 (PIA), respectively, have low toxicity to rats exposed by the inhalation route. The green dye mixture produced a mild inflammatory response in the lung parenchyma, and this dye is also cleared much more slowly from the lung than is the yellow dye. The dye components of the developmental red and violet smoke mixture for the M18 grenade have been subjected to a series of inhalation toxicity assays similar to those performed upon the yellow and green smoke dyes. These studies show the developmental violet dye mixture to be acutely toxic to the liver and nasal epithelium in rats, while a 13-week series of daily 6-hour exposures of rats to the red dye mixture produced only a subtle and mostly reversible restrictive lung disorder without associated pathology. Because of the high level of toxicity of the developmental violet dye mixture in rats, USABRDL has recommended that the developmental niixture not be used in the M18 grenade. This has led to a reconsideration of the need for a violet smoke grenade. Data from in vitro testing of the dyes in the product-improved yellow and green grenades and in the develcpmental red and violet grenades show generally a weak, but significant potential to cause genetic damage. [21]

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G. Graphite

1. General Information

a. Bulk Material

The military uses two synthetic graphite powders, Micro-260 (manufactured by the Asbury Graphite Mills, Inc.), and KS-2 (manufactured by Dixon Ticonderoga Company). These graphites are composed of platelets or flakes of various sizes. In a recent study of particle size analysis of graphite from an XM56 generator, the Mass Median Diameter (MMD) for Type 1 and Type 2 Reprocessed IR material were 3-5 microns and 47-106 microns, respectively.[1] The chemical composition of the bulk powders is predominantly carbon with trace impurities totaling <1% by weight. The trace impurities include small quantities of silica, aluminum, iron, calcium, titanium, and magnesium.

b. Aerosol Composition/Dispersion

Graphite flakes become disseminated to the environment from ground-based systems by the mechanical dispersion of bulk powders into the atmosphere. The powder is used directly or compressed into small pellets to improve handling and delivery to the air-ejector of smoke generators. Because the aerodynamic sizes of air-dispersed flakes are small, near-source surface deposition caused by particle settling is limited. Near-source deposition can be significant if the air-ejector is oriented at or near parallel to the ground. The long-range downwind patterns resulting from dispersion and deposition depend on local meteorlogicial conditions.

2. Pharmacokinetics/Metabolism

Graphite dust behaves biologically as "nuisance dust", producing little adverse effect when exposures are kept under control. The pulmonary response to nuisance dusts is characterized by accumulation of dust-laden macrophages in the alveoli, perivascular tissue, and bronchiolar region of the lung, as well as, the proliferation of Type II pneumocytes. No deposition of collagen fiber or alteration of stromal lung structure is observed. Exposures below 10 mg m^{-3} are unlikely to result in disease during the working lifetime of an individual. Because the presence of crystalline silica in graphite and graphite products appears to increase fibrogenic potential, occupational exposure standards are currently based on the silica content of the dust.[2]

3. Health Effects

a. Skin and Eye Effects

Deposits of graphite flakes (as with any particulate matter) in eyes, ears, and nasal passages may cause discomfort.[3] However, rabbit eye/skin irritation tests with graphite were negative, and cutaneous toxicity studies in rabbits indicated that graphite has no effect via this route.[4]

b. Inhalation Effects

Graphite flakes pose the greatest risk to humans through inhalation. Accumulation of graphite within the body produces a pneumoconiosis that in its most simple form is characterized by focal collection of dust-laden macrophages at the division of the respiratory bronchioles, reticulin deposits, and focal emphysema. Progressive forms of macular and nodular lesions to massive fibrosis with "graphite cysts" have also been observed.

The fibrotic constituents of graphite mixtures may screen the etiology of pneumoconiosis from graphite. Composition analysis of some graphite mixtures have shown to contain from 2% to 25% free silica. Acute exposure studies with rats have demonstrated that graphite without contaminating silica produced minimal, reversible pulmonary effects.[5,6] The effects included enzymatic and cytological alterations, and changes in pulmonary resistance, all of which were resolved within 14 days.[2] Although the occupational exposure standard adopted by the ACGIH to protect against silica-induced fibrosis is 0.1 mg m^{-3} respirable quartz, more than 44% of employees exposed to synthetic graphite dust containing <0.1 mg/m^3 free silica exhibited pneumonoconiosis.[7] The ACGIH has issued a notice of intended change for the graphite exposure standards what will limit occupational exposure to all forms of graphite (except fibers) to 2 mg m^{-3} of the respirable fraction.[8]

c. Reproductive /Teratogenic Effects

No information is available concerning reproduction studies.

4. Summary

Typically air suspended particles of greater than resirable diameter and which contains less than 1% crystalline silica causes little or no adverse effects if kept under control. Discomfort from reduced visibility or deposition in nasal passages, etc. or with dermal contact may degrade safe work practices or quality of work.

Further examination of soldiers' exposure is in order, especially with the employment of the XM56 smoke generator screening equipment, presently being fielded.

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H. Titanium Dioxide

1. General Information

a. Bulk Material

Titanium dioxide is generally classified as a "nuisance dust" with a threshold limit value (TLV) of 10 mg/m^3 of total dust (<1% quartz).[1] It is the proposed major component for the developmental training grenade XM82.[2]

Three TiO_2 dusts from different sources were tested and found to contain approximately 97% TiO_2 and variable amounts of amorphous silica, alumina or aluminum oxide (<2%), and siloxane hydrophobic coating (<0.5%).[3]

b. Pharmacokinetics/Metabolism

Toxicity and fate studies were performed using Daphnia magna to decide impact of TiO_2 on the environment. The titanium dioxide materials employed did not show any apparent toxic effects to daphnia up to 1000 mg L^{-1}. Microscopic examinations showed that the Daphnia ingested the materials and passed it through the gut with no apparent internal damage in 48 hrs.[2]

3. Health Effects

a. Skin and Eye Irritation

There is no data available to determine skin or eye irritation.

b. Acute Inhalation Exposure Effects

Respiratory physiological testing and bronchoalveolar lung lavage was performed on 344 Fischer rats 24 hours and 14 days post-exposure to TiO_2. Parameters for pulmonary function and impairment test included respiratory flow and transpulomonary pressures for each animal. Once the pulmonary measurements were completed bronchoalveolar lavage was performed using 0.015 mL of saline per gram body weight for each rat. Lavage fluid was aspirated and centrifuged at 300 g for 10 minutes at 4°C. The supernatant was assayed for total protein and enzymatic activity of lactate dehydrogenase (LDH), alkaline phosphatase (ALKP), and B-Glucuronidase (B-Glu). (Table 2)

Male Fischer 344 rats were exposed to combustion gases from a quarter-size grenade of TiO_2 detonated in a 20-m^3 chamber. Exposures were delivered via (polyvinylchloride) pipe into an adjacent "nose-only" chamber that restricted movement of the rat, but ensured a 500 L min^{-1} exposure for each rat.

The rats were exposed to a low, mid, and high concentrations of TiO_2 for 30 minutes but did not exhibit any adverse toxic effects. The administration of explosively disseminated TiO_2 dust resulted in the deposition of brown granular pigment in the lungs of all exposed rats. The pigment was contained within macrophage. No toxic or inflammatory reactions, other than phagocytosis of the dust particles in the lung, were present. Pigment-laden macrophage were also present in the lungs of all rats in the fuse/fuel-exposed control group. There were no other significant gross or histopathologic differences between the animals in the 24-hour and 14-day PE groups.[2]

c. Repeated Inhalation Exposure Effects

Although no adverse toxicological effects were shown in this study during short-term, high-level exposures, repeated dust exposures, according to the American Conference of Governmental Industrial Hygienists, should be kept below the nuisance dust level of 10 mg m^{-3}.[1]

d. Carcinogenic Effects

Presently, there have been no studies suggesting the carcinogenicity of TiO_2 in test animals or humans.

4. Summary

Titanium dioxide has been used in various industrial applications from paint, paper, plastics, ceramics, inks, and floor coverings. Employees occupationally exposed to TiO_2 for over 10 years have showed no evidence of adverse pulmonary effects.[4] Its proposed use as a training grenade, barring additional evaluation, is under developmental investigation by the U.S. Army Chemical Research, Development and Engineering Center (CRDEC).

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ABBREVIATIONS

µmmicrometer
ggram
mgmilligram
MMADMass Mean Aero Diameter
ACGIHAmerican Conference of Governmental Industrial Hygienist
DF-2Diesel Fuel 2
VEESSVehicle Engine Exhaust Smoke System
USEPAUnited States Environmental Protection Agency
HPLCHigh Pressure Liquid Chromatography
DCPBdecachlorobiphenyl
σSigma
mMeter
lLiter
LC50Letal Concentration (50%)
RD50Response Dose (50%)
SGFSmoke Generator Fuel
m^3Meter Cubed
PAHPolycyclic Aromatic Hydrocarbon
NOAELNo Adverse Effects Level
TWATime Weighted Average
EEVEnd Expiratory Volume
AHHAryl Hydrocarbon Hydroxulase
ppmparts per million
ppbparts per billion
LCELoad Carrying Equipment
DNADeoxyribonucleic Acid
SV7Simian Adenovirus 7
WP White Phosphorus
NIOSHNational Institute for Occupational Safety and Health
MOUTMilitary Operations on Urban Terrain
RPRed Phosphorus
BRButyl Rubber
BUNBlood Urea Nitrogen
APDAlkaline Phosphatase Dehydrogenase
OECDOrgainization for Economic Corporation and Development
PAMPulonary Alveolar Macrophages
PTA1,4-di-p-toluidino-9,10-anthraquinone
VDMViolet Dye Mixture
DRDisperse Red
SGPTSerum Glutamic-Pyruvic Transaminase
USABRDLUnited States Army Biomedical Research and Development

Laboratory
ALKPAlkaline Phosphatase
Beta-Glucuronidase
CRDECU.S. Army Chemical Research Development and Engineering Center

CHEMICALS

ZnZinc
ClChloride
HCHexachloroethane
TiTitanium
O_2Oxygen
CCl_4Carbon Tetrachloride
AlAluminum
NNitrogen
NO_xNitrogen Oxides
QID(2'-quindyl)-1,3-indandione